

FABRICATION AND PERFORMANCE OF AMMONIUM ION-SENSITIVE GLASS ELECTRODES

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for the Degree of
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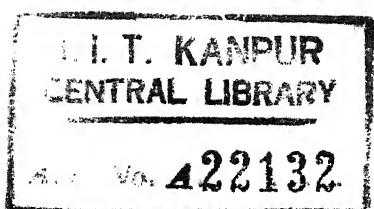
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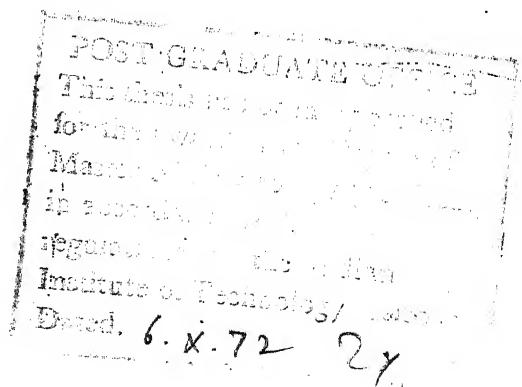
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CERTIFICATE

This is to certify that this work has been carried out under my supervision and has not been submitted elsewhere for a degree,

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NOMENCLATURE

a_I	= activity of the ion I
a_{int}	= activity of the interfering ion
$C_{NH_4^+}$	= concentration of ammonium ions in the test solution, moles/liter
E_{cell}	= e.m.f. of the electrochemical cell, millivolts
E_{ind}	= indicator electrode potential, millivolts
E_{ref}	= external reference electrode potential, millivolts (assumed to be constant in a properly designed system)
E_j	= liquid junction potential, millivolts (assumed to be either negligible or constant for a particular system)
E_{const}	= $E_{ref} + E_j$
E°	= constant standard potential for the indicator electrode, millivolts
E°	= $E^{\circ} + E_{const}$
E	= same as E_{cell}
F	= Faraday constant
I	= ion to which the indicator electrode is selective
k	= selectivity coefficient
M	= molarity of the test solution (moles/liter)
n	= charge on the ion I
R	= gas constant
T	= absolute temperature
z	= charge on the interfering ion.

ABSTRACT

A method for the fabrication of five ammonium ion-sensitive glass electrodes using different glass compositions, internal filling solutions and internal reference electrodes is described and their performances are reported. All the electrodes are found to give a Nernstian response to NH_4^+ in the concentration range 1.0 to 0.0001 M and experimental Nernst slopes agree within 5.19% of the theoretical value at 28°C. However, the response rates of all the electrodes are observed to be extremely slow and dependent on the direction of the concentration jump and ammonium ion concentration in the test solution.

All the electrodes were used for the determination of urea via urease-catalyzed hydrolysis of urea to NH_4^+ and an accuracy of 5.00% was obtained.

For the effect of soaking media on the response times of the electrodes, it is found that pretreatment of the glass electrode in a medium similar to that it is to be used in, gives a faster response compared to preconditioning in four times distilled water.

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CHAPTER 1

INTRODUCTION

Ion-selective electrodes are defined simply as those electrode devices which selectively measure the activity of one given ion or of a series of such ions in an aqueous or nonaqueous solutions of different ions.

An ammonium ion-sensitive glass electrode should be useful wherever a direct measurement of ammonium ion activity is desired, whether this be in a simple solution or in a complex ionic mixture. The general areas of application in which this sensor can be used include solution chemistry (for example, analytical and biochemistry), biomedical research, clinical measurements, water pollution control, and quality and process control.

The specificity of enzymes, and their ability to catalyze reactions of substrates at low concentrations is of great use in chemical analysis. Enzyme catalyzed reactions have been used for analytical purposes for a long time for the determination of substrates, activators, inhibitors, and also of enzymes themselves.

One of the main objections generally raised, regarding the use of enzymes in chemical analysis, is the high cost of these materials (enzymes and some of the substrates). A continuous or semicontinuous routine analysis using enzymes would

require significant amounts of these materials, quantities greater than can be supplied. Such large quantities of these materials represent a large expenditure which may be prohibitive in a number of cases. If, however, the enzymic analysis is performed by heterogeneous catalysis at an electrode surface by coupling either enzyme or substrate to the surface of the electrode, as the case may be, very small amounts of enzyme and substrate should be required. The amount required would be limited by the area of the electrode, thus making the analysis cheaper. The cost of enzymic analysis will be further reduced, if one employs insolubilized form of the enzyme (without loss of activity for atleast a reasonable period of time), because in that case the same sample of immobilized enzyme can be used continuously for many hours.

Ammonium ion-sensitive glass electrodes can be used for the preparation of enzyme-substrate electrodes which in turn can be used for the determination of various enzymes or their corresponding substrates. These electrodes make use of the ability of an enzyme to react selectively with a complex organic molecule to produce ammonium ion, which is then sensed by the electrode. The enzyme electrode is prepared by immobilizing a layer of the enzyme in polyacrylamide gel over the surface of the glass electrode responsive to ammonium ions. The ammonium ions produced in the enzyme catalyzed substrate hydrolysis reaction diffuse through the immobilized gel layer and are sensed by the electrode. The resulting e.m.f. is proportional to the substrate

concentration in the test solution. With certain modifications, this electrode system can be reversed, that is, with the substrate coupled to the ammonium ion-sensitive glass membrane, resulting in an enzyme-sensing electrode.

The usefulness of an ammonium ion-sensitive glass electrode in determination of the activity of deaminase enzymes and the concentration of their corresponding substrates, in studying the kinetics of deaminase enzyme systems (urease, asparaginase, glutaminase, amino acid oxidase, and amine oxidases), and in the direct measurement of urea in blood and urine, has already been established (1,2).

The use of such electrodes for analytical purposes offers unique advantages over the other conventional methods, when non-destructive, rapid, and continuous recording of ionic activity with high sensitivity is desired.

A unique characteristic of these sensors is that they sense ionic activity, which in a large number of cases is more physiologically and physicochemically meaningful than concentration. Whenever, one is concerned with the rate of a chemical reaction or the position of an equilibrium, the activities of the reacting species are the parameters of interest. Although, these electrode measure ionic activities, they can also be used to determine free ion and total concentrations through methods such as titration, standard addition, and ionic strength buffering.

Electrode measurements are very fast—almost instantaneous in many cases. Generally, response times are of the order of

fraction of a second under proper conditions. Even under unfavourable conditions, it is possible to obtain a reading within two minutes. They, therefore, find ready application in such areas as chemical kinetic studies and process control. Instead of batch sampling and analysis, these electrodes can be built into the process stream, that is, on line monitoring, for continuous readings of selected species.

The electrode is virtually non-destructive of the sample. The number of ions transported across the membrane to establish the equilibrium e.m.f. is very small compared to the number of ions present even in the extremely dilute test solutions. Therefore, with an appropriate electrode geometry, even samples amounting to a few tenths of a milliliter can be handled. These advantages can be very important in clinical measurements, where several determinations must be performed on a single small sample.

With the use of ion-selective electrodes, in most cases, pretreatment of the test solution is not required. Time consuming operations for sample pretreatment in other conventional methods are eliminated in this case.

Finally, when compared to most other instrumental methods of analysis, the equipment required for ion-selective electrode studies is quite simple and relatively inexpensive.

In the present study, five ammonium ion-sensitive glass electrodes were fabricated and tested for their response towards

ammonium ions and their applicability in urea determination. Dynamic response of all the electrodes to various concentrations of ammonium ions and to the reaction product (NH_4^+) of urease-catalyzed hydrolysis of urea were investigated. Nernst response was plotted from the steady state e.m.f. values obtained under dynamic response studies. Effects of glass composition, internal filling solution, internal reference electrode, ammonium ion concentration, direction of concentration jump, and soaking media on the electrode response were determined. Lastly, all the electrodes were tested for their stability.

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CHAPTER 2

LITERATURE REVIEW

Interest in electrodes selective, or specific, to certain ions dates back atleast to the turn of the century and most of the developments to be described below can be traced back to Cremer(3). In 1906, Cremer found that an electrode made from a glass bulb changed its potential when immersed in solutions having different hydrogen ion concentrations. Three years later, in 1909, Haber and Klemensiewicz(4) demonstrated that this glass electrode potential was dependent on the hydrogen-ion concentration in the same manner as the potential of a hydrogen electrode.

In 1922, Hughes (5) made the first observations indicative of the existence of a glass electrode that could respond to species other than H^+ , when he established that the electrode potential deviated from the Nernst equation in alkaline solutions, noting that the more Al_2O_3 the glass contained the larger was the deviation, that is, the less Al_2O_3 the glass contained the better it was for measuring H^+ . Horovitz (6,7) and Schiller (8) soon afterward showed that the introduction of B_2O_3 caused glass electrodes to become nearly as sensitive to sodium ion as to hydrogen ion. These observations were extended by Lengyel and Blum (9), who in 1934, conclusively reported that the addition of Al_2O_3 or B_2O_3 to sodium silicate glasses caused potentials of electrodes to become strongly dependent upon Na^+ , as well as H^+ . These authors were the first to show that one

could thereby obtain a Nernst response to sodium ion.

Eisenman, Rudin, and Casby (10) took up this point and in 1957 presented evidence that, indeed, all sodium silicate glasses containing more than a fraction of 1 mole percent of Al_2O_3 were markedly cation-sensitive. What was only an objectionable error in a pH electrode was transformed into a useful characteristic by decreasing the relative affinity of the electrode for H^+ and increasing its affinity for alkali-metal cations. By a systematic study of simple glasses containing only Na_2O , Al_2O_3 and SiO_2 , they showed that the sensitivity for the alkali-metal cations relative to one another and to H^+ was a systematic and reproducible function of glass composition. Eisenman et.al.(10) also characterized those ranges of glass composition which had sufficiently high selectivities for Na^+ or K^+ to be of practical use for the measurement of Na^+ , on one hand, or K^+ , on the other. This work was considerably extended by Eisenman et.al. (11-13), Nicolskii et.al.(14-17) and by Shultz et.al. (18-25) and was the starting point for present studies on glass electrodes selective for particular cations.

Although, the work of Eisenman et.al. (10) was primarily concerned with the ternary glass system of the sodium alumino-silicate series, they pointed out that similar cation-sensitive systems could be made with lithium or potassium in place of sodium, with boron in place of aluminium and germanium in place of silicon. One could refer to all of these glasses collectively as cation-specific.

2-1 AMMONIUM ION-SENSITIVE GLASS ELECTRODES.

Hattock and Uncles (26), investigated the properties of an electrode glass, designated BH115, and found that this glass showed a selective response to potassium and ammonium ions. The working range for response to both types of ions was found to be from 1.0 to 0.0001 M. The response was Nernstian and the slope was in good agreement with that predicted from the Nernst equation. The effect of hydrogen ions, sodium ions, and ions of the alkaline-earth elements on the response were determined. Only sodium ions exerted significant interference. The response of BH115 electrode to ammonium ions was slightly superior to its response to potassium ions. Reproducibility, stability and speed of response of the glass was comparable to those of pH electrodes.

Eisenman (27,28) reported that the composition of a typical ammonium ion-sensitive glass was NAS27-3+3 mole% ZnO (27 mole% Na₂O, 3 mole% Al₂O₃, 67 mole% SiO₂ and 3 mole% ZnO). This glass composition was found to give reproducible Nernst response to ammonium ions. Eisenman (27,28) pointed out that the selectivity of glass for ammonium ion was a systematic function of composition representable in terms of Na-K selectivity. With Na⁺ and H⁺ as the principal contaminants and K⁺ concentration sufficiently low, he observed that any K⁺-selective electrode would be satisfactory for measuring NH₄⁺.

It was reported that cation-sensitive glass electrodes

also responded to a variety of substituted ammonium ions, amines and amino acids (28).

Guilbault et.al. (1) found the Beckman 39137 cationic electrode having sensing tips comprised of specially formulated glass with composition 27% Na_2O , 4% Al_2O_3 , and 69% SiO_2 , and marketed by Beckman Instrument Co., Fullerton, California, U.S.A. to respond to ammonium ions in the concentration range 10^{-1} to 10^{-5}M with a deviation of ± 0.5 mV in the 10^{-1} to 10^{-4}M region and $\pm 20\text{mV}$ at 10^{-4} to 10^{-5}M region. The selectivity order of this electrode as reported by the authors is $\text{Ag}^+ > \text{K}^+ > \text{H}^+ > \text{NH}_4^+ > \text{Na}^+ > \text{Li}^+ > \text{Mg}^{2+}, \text{Ca}^{2+}$ and that reported by the manufacturer is $\text{H}^+ > \text{Ag}^+ > \text{K}^+, \text{NH}_4^+ > \text{Na}^+ > \text{Li}^+ >> \text{Mg}^{2+}, \text{Ca}^{2+}$.

Csakvari et.al.(29) and Lengyel et.al. (30) also worked on electrodes sensitive to ammonium ions.

A liquid membrane electrode sensitive to ammonium ions was reported by George Baum (31) and Simon (32). Cosgrove et.al(33) developed a new NH_4^+ - selective electrode that was capable of measuring ammonium ions in the presence of other cations. Cammann (34) described a glass electrode for measuring NH_4^+ and other monovalent cations.

In 1971, Barica (35) reported the use of a univalent cation glass electrode for determining ammonium in water. The method enabled direct determination of NH_4^+ in concentrations $> 0.5 \text{ mg/l}$ without any dilution or pretreatment of sample.

2-2 APPLICATION OF AMMONIUM ION-SENSITIVE GLASS ELECTRODES IN BIOCHEMICAL ANALYSIS AND CLINICAL MEASUREMENTS.

Katz and Rechnitz (36,37) used the cation-sensitive glass electrode responsive to ammonium ions for the determination of urea and urease via complete conversion of urea to NH_4^+ by enzyme catalyzed hydrolysis. The e.m.f. measured in the solution after hydrolysis was proportional to the amount of NH_4^+ produced and hence to the concentration of urea and urease. They found that a continuous measurement of NH_4^+ liberated was possible in this case.

Montalvo (38,39) developed an electrode for the analysis of urease via catalytic decomposition of urea at the active surface of a cationic electrode responsive to NH_4^+ . The electrode was made by coupling urea to the active surface of the cationic electrode. The electrode performed well, indicating that development of electrodes utilizing more expensive substrates would be in order.

A urea-sensitive electrode was described by Guilbault and Montalvo (40-43) for the determination of urea in aqueous solutions. The electrode was prepared by immobilizing urease in a layer of acrylamide polymer on the surface of a Beckman cationic electrode sensitive to ammonium ions. Concentrations as low as 10^{-6} M urea could be detected. By placing a thin film of cellophane over the immobilized gel layer, the electrode could be used continuously at 25°C for three weeks with no loss in activity.

Guilbault et.al. (1) investigated the application of cation electrodes to a study of the kinetics of deaminase enzyme systems. Guilbault and Hrabankova (2) reported a urea-sensitive electrode for the direct assay of urea in blood and urine. An accuracy of about 2-3% was obtained. Cosgrove (33) and Simon (32) used a new NH_4^+ -selective electrode to analyse blood serum samples for urea content by treatment with urease without any special sample pretreatment.

Guilbault and Shu (44) prepared an enzyme electrode specific for glutamine by entrapping glutaminase on a nylon net between a layer of cellophane and a Beckman 39137 or Thomas 4923-Q10 cation electrode. The electrode responded to glutamine over the concentration range 10^{-1} - 10^{-4} M with a response time of only 1-2 minutes. The electrode could be used continuously for about 12 hours.

In recent years, a large number of reviews (32,45-62) on glass membrane electrodes, homogeneous and heterogeneous solid-state membrane electrodes, liquid ion-exchange membrane electrodes, enzyme electrodes and antibiotic electrodes have appeared.

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CHAPTER 3

THEORY OF ION-SELECTIVE GLASS ELECTRODES

There are three fundamentally different types of membranes which selectively interact with the ion or ions to be determined:

1. glass membrane

2. solid-state membrane:

(a) homogeneous solid-state membrane

(b) heterogeneous solid-state membrane

3. liquid membrane:

(a) electrically charged ligand groups (ion-exchangers)
as membrane components

(b) electrically neutral ligand groups as membrane
components.

Since, glass membrane is the typical representative of these systems, the theory of only glass membrane electrodes will be discussed here.

3-1. PROPERTIES OF GLASS.

A. Conduction of Electricity in Glass:

For measuring the e.m.f. of a cell with high accuracy, the resistance of the measuring circuit must be very much greater than that of the cell. Hence the resistance through the glass bulb or membrane which forms the glass electrode must be less than that of the measuring instrument. In practice the specific resistance of the glass should not normally be larger than about

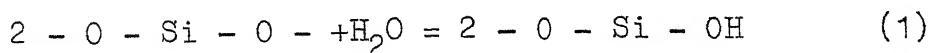
10^{12} ohm-cm. Since the resistivity of glass varies widely with composition and may reach very high values, especially at room temperatures, it limits the range of glass compositions which can usefully be employed as electrodes.

Electrical conduction in sodium silicate glasses is electrolytic, the current being carried exclusively by the sodium ions moving through the immobile anionic network. No single cation moves through the entire thickness of the dry glass membrane but, rather, the charge is transported by an interstitial mechanism by which each charge carrier only needs to move a few atomic diameters before passing on its energy to another carrier.

The simple alkali silicate glasses are relatively highly conducting and the conductivity increases with the alkali content, that is, with the concentration of mobile ions. The substitution of Al_2O_3 for SiO_2 in soda silica glasses lowers the activation energy and raises the conductivity, as long as the atomic ratio Al/Na is less than unity (63); for Al/Na greater than 1, the conductivity decreases again with Al_2O_3 content.

B. Effect of Hydration of Glass:

When glass is placed in water, some of the Na^+ ions in the glass are exchanged by H^+ ions from the water (28). Doremus (64) writes that when glass is treated with water, silicon-oxygen bonds at the glass surface are broken and hydroxyl groups are formed, as described by the reaction



The reaction is written in this way to show that the hydroxyl groups need not form on the same silica group; in fact, they probably are formed randomly.

When the glass is first immersed in water or aqueous solution, a hydrated layer is formed at the surface, causing some swelling of the silica network. After the initial hydration a constant dissolution of the hydrated layer takes place, with further hydration of additional dry glass. In the steady-state, the thickness of the hydrated layer is maintained at some approximately constant value.

The resulting "hydrated" glass can have properties quite different from the dry glass. Hydration may facilitate the movement of ions in the glass, lowering the electrical resistance to practical levels. The diffusion of ions in highly hydrated soda aluminosilicate glasses is reported to be 100-1000 times higher than in the dry glass (64), although the equilibrium properties of ion-exchange are not so greatly changed by hydration. The structure of the hydrated glass and how it differs from the dry glass remains a puzzle.

Before the glass electrodes are put in service, they must be soaked in deionized or triply distilled water for a long time to give a hydrated layer of sufficient thickness to provide uniform conditions for ionic interdiffusion (28).

The practice of storing electrodes in distilled water or buffer is based on the conclusion that a hydrated gelatinous

surface layer is essential for the generation of a boundary potential at the glass-solution interface (28).

C. Ion-Exchange Properties of Glass:

A membrane made of glass is permeable almost solely to cations and functions as a cation exchanger. Evidence for the cation - exchange properties of glass is ample (27).

Silicate glasses consist of a network of silicon-oxygen bonds; at temperatures below the softening point this network is very rigid. If some sodium oxide is included in the glass melt, some silicon-oxygen bonds are broken and ion groups with singly charged oxygen ions (negative) and positive sodium ions are formed. In the cooled, rigid glass the oxygen ions are held in place by the silicon-oxygen network, but the sodium ions are quite mobile and can diffuse readily through the silicate structure and can exchange with other ions in a solution at the glass surface. These mobile monovalent ions give the glass its ion-exchange character (28, 64). The exchange is normally between monovalent cations in the source solution and the sodium ions in the glass, and is one for one. Cations of higher valency than one are much less mobile in the silicate network, although there are reports of the sensitivity of certain glass electrodes to divalent cations (28, 64).

3-2. THE GLASS-ELECTRODE POTENTIAL.

A. Origin of the Glass-Electrode Potential:

The origin of the glass-electrode potential has been a subject of some dispute. There were in general two schools of thought (28). In one view, the potential was supposed to arise exclusively as a phase-boundary potential at the membrane-solution interfaces; the other view argued that it arose within the interior of the membrane as a diffusion potential. In recent years, these views have been satisfactorily synthesized (28), and it now seems virtually certain that the glass electrode is simply a perfect cation exchange membrane; and the glass electrode potential originates as a result of ion-exchange processes, and is the sum of diffusion and phase-boundary potentials. The phase-boundary potential arises because of a difference in activity of an exchangeable ion between the solution and exchanger phases, while the diffusion potential results from differences in ionic mobilities within the exchanger.

Thus, when a thin membrane of glass is interposed between two solutions, an electrical potential difference, the so called glass electrode potential, is observed across the glass. This potential can be measured by making electrical contact to the inner solution with a suitable internal reference electrode, and at the same time contacting the external (sample) solution with a second reference electrode (external reference electrode) via a salt bridge, and connecting the leads of the

two reference electrodes (internal and external) to a high input impedance voltmeter, as shown in Figure 1.

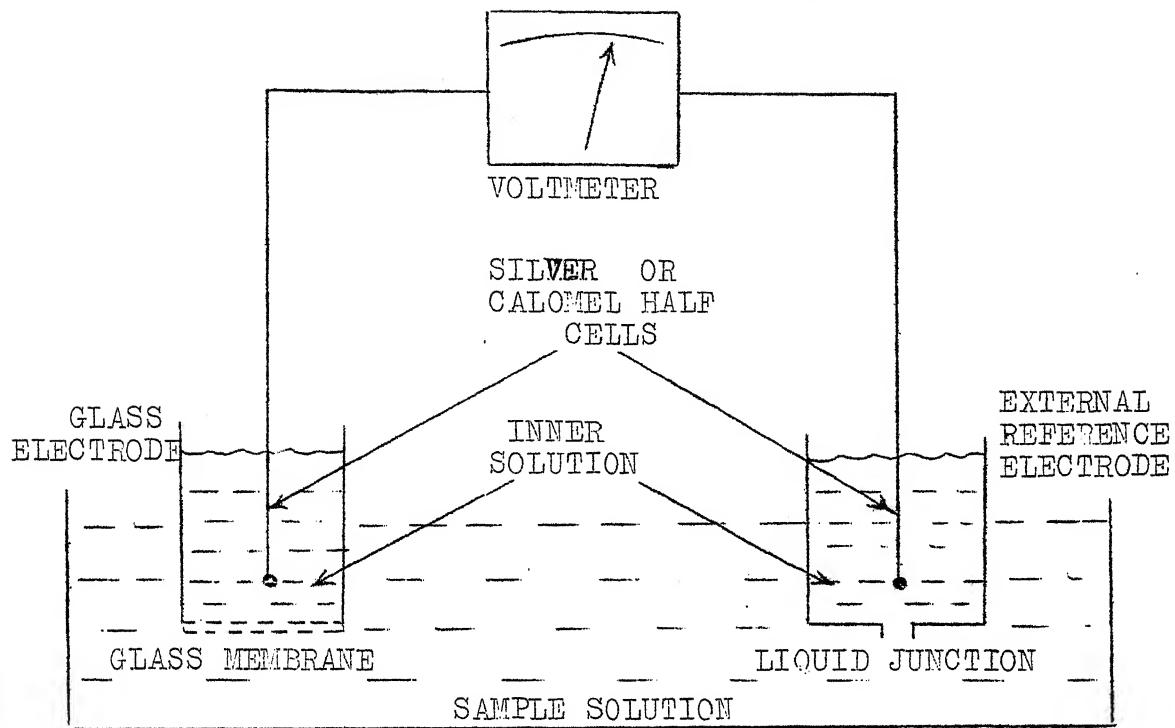


Figure 1. Schematic Diagram of Cell Used to Measure Electrical Potentials Across Glass Membranes

For a complete electrochemical cell, the e.m.f. is given by:

$$E_{\text{cell}} = E_{\text{ind}} + E_{\text{ref}} + E_j \quad (2)$$

where E_{ind} , E_{ref} , and E_j are the indicator electrode, external reference electrode, and liquid junction potentials, respectively. (The indicator electrode is the sensing glass electrode, and the liquid junction is an interface between dissimilar solutions.)

Ideally, the reference electrode is of invariant potential with negligible variation in the liquid junction potential from one test or calibration solution to another. Therefore, in a properly designed system, E_{ref} is constant and E_j is either constant or negligible. Equation (2) then becomes

$$E_{cell} = E_{ind} + E_{const} \quad (3)$$

If an indicator glass electrode is constructed by filling a thin walled glass bulb with a solution of a salt of constant composition, and if it is completely selective towards an ion I^- , then its potential would be given by the Nernst equation,

$$E_{ind} = E^{\circ} + (RT/nF) \ln a_I \quad (4)$$

where E° is the constant standard potential for the indicator electrode; R, T, F, and n are the gas constant, absolute temperature, Faraday constant, and charge on the ion I^- , respectively; and a_I is the activity of the ion I^- to which the electrode responds. By substituting the equation (4) into equation (3) one obtains

$$E_{cell} = E^{\circ} + (RT/nF) \ln a_I + E_{const} \quad (5)$$

If the E_{const} term is combined with E° , the observed cell potential is

$$\underline{E}_{cell} = \overline{E}^{\circ} + (RT/nF) \ln a_I \quad (6)$$

where $\overline{E}^{\circ} = E^{\circ} + E_{const}$. Thus it is seen that the cell potential varies directly with the logarithm of the ionic activity or concentration. In the case of less - than - perfect electrode

specificity, an additional term is added to account for such an electrode interference (62):

$$E_{\text{cell}} = \overline{E^{\circ}} + 2.303(\text{RT}/n\text{F}) \log(a_I + k a_{\text{int}}^{n/z}) \quad (7)$$

where k is the selectivity coefficient and a_{int} is the activity of the interfering ion of charge, z .

From equation (6), it is clear that for a glass electrode to be sensitive to ammonium ions, a plot of E_{cell} vs. logarithm of NH_4^+ concentration should give a straight line with a slope of $2.303 \times (\text{RT}/\text{F})$, when E_{cell} is measured in solutions containing ammonium ions only.

A detailed discussion on derivation of equation for electrode potential can be found elsewhere (27,28,62,64).

B. Time Variation of Potential:

Glass electrodes often show variations of potential with time when taken from one solution of constant composition and placed in another. In their simplest form, the theories based on ion-exchange equilibrium cannot yield time-varying terms. Both the diffusion potential and the interfacial potential are independent of time, provided equilibrium is established between the solution and the surface layer of glass. Isard(28) has reported that if the properties of the ion-exchange sites are modified as a result of the ion-exchange, time variations of potential can arise. The most important effect is probably that of hydration. For example, if a soda silica glass exchanges its sodium ions for hydrogen ions, the ion-exchanged layer may take up

water molecules leading to swelling; hence the values of the equilibrium reaction constants at the surface may vary with time, and therefore, the glass electrode potential.

According to Rechnitz (28), two effects seem to be involved in the over-all rate of re-equilibration of a glass electrode in response to a change in solution composition. The first of these might be termed the 'rate of reconditioning' and has for its physical basis the replacement of ions in the hydrated gel layer of the electrode. This effect would be predominant when the electrode encounters a sample composed of ions other than those provided during the original conditioning (soaking) of the electrode. Because the movement of ions into the gel layer takes place largely by diffusion, it is not surprising that glass electrodes should show sluggish response characteristics and "drifting" in response to changes in the ionic nature of their environment. The same type of process may also be responsible for the puzzling e.m.f. transient often encountered when a highly specific electrode is exposed to a sample containing a mixture of cations.

The true response rate of glass electrodes is perhaps more properly defined as the rate of re-equilibration of an electrode, initially at steady state, in response to a step change in the concentration of a single potential determining ion in a medium consisting of a pure solution of a salt of that ion. Since the e.m.f. of a glass electrode at steady

state subjected to such a change is dependent only upon the ion-exchange equilibrium at the gel layer-solution interface, such response rates can be expected to be rapid.

Savage and Isard (65) have mentioned that the final steady values of potential have been used to relate electrode measurements to the ion-exchange properties of the glass (that is, equilibrium reaction constants) as if the observed drifts of potential were due to the slow attainment of chemical equilibrium at the interface. However, it seems very unlikely that the cationic exchange between a solution and the glass network would require times of upto one hour to reach equilibrium potential. Further the time to reach equilibrium potential is of the order of seconds or less for an H⁺ sensitive sodium silicate glass and of the order of an hour for a cation sensitive sodium aluminosilicate or sodium boro-silicate glass. It may be noted that the ionic mobilities are greater in the aluminosilicate glasses, as judged from their electrical conductivities, showing that there is no simple relation between the time constant in electrode measurements and the ionic diffusion rates.

Following Savage and Isard (65), the most probable general explanation of the potential drifts is that they are associated with changes in the degrees of hydration of the ion exchanged layer, for the total reaction of glass with aqueous reagents cannot be represented by the simple ion-exchange equation in which the anionic glass network is regarded as inert.

The exchange of cations from the glass by hydrogen ions from the solution will, in general, be accompanied by a hydration reaction which may not be rapid. This hydration of the network may alter the ionic mobilities and hence changes of potential will accompany any changes of hydration. Certainly the time effects depend on the initial glass compositions used and the immersion history of the particular electrode and tend to be more pronounced where the glass is not fully responsive to a single cation.

Surprisingly little information is available in the literature regarding the response rates of cation-sensitive, or even pH-type, glass electrodes.

C. Eisenman's Theory of Glass Electrode Selectivity:

The selectivity of glasses for ions has a definite dependence on the composition of the glass. The most important factor in this dependence is the type of anionic site in the glass (SiO_4^{4-} site in the sodium silicate glass and AlOSi_3^{4-} site in the sodium aluminosilicate glasses). Eisenman (13) places particular emphasis on the electrostatic energies or "field strength" of the sites available within the glass structure for cationic exchange with the solution phase. It is clear from his treatment that hydrogen ion-selective glasses and alkali ion-selective glasses may be regarded as extreme members of a continuous series of cation - responsive electrodes.

The glass lattice consists essentially of tetravalent Si^{4+} atoms coordinated with oxygen atoms to form a fairly fixed structure. Throughout the structure one finds Na^+ ions in the vicinity of the oxygens of the silica tetrahedra. The latter are exchangeable for H^+ and indeed, it has been found that such a sodium silicate will bind H^+ about 10^{14} times as strongly as Na^+ at equilibrium. Such a glass is therefore H^+ sensitive. Aluminium atoms introduced in small numbers into the glass can, like Si, exist in tetrahedral coordination with the oxygen atoms. Such alumina tetrahedra must, however, carry an excess negative charge since Al^{3+} replaces Si^{4+} . The excess negative charge can be screened in part by whatever Na^+ is available within the glass, but such screening is not completely effective. Accordingly, the interaction of cations (including H^+) will be stronger in a sodium aluminosilicate than in a sodium silicate by the amount of excess electronic charge that is not effectively screened. Similarly, since the binding energies of cations to alumina tetrahedra will be so much higher than to silica tetrahedra, equilibrium will be dominated by interactions with the former in a glass containing even a small amount of aluminium. It is perhaps for this reason that the onset of major ion specificity occurs abruptly with the addition of even small amounts of aluminium.

The selectivity of cation-specific glasses for one alkali metal cation relative to another is a systematic and reproducible function of the glass composition and appears to be

basically dependent on the sodium to aluminium ratio of the lattice (10). Eisenman (13) explained the variation of selectivity as a function of composition in the sodium aluminosilicate glasses as resulting from "screening" of the AlOSi^- sites by sodium ions on SiO^- sites. When the ratio of sodium to aluminium ions is less than 1, the extra aluminium ions go into the glass structure as network - breaking ions rather than as substitutes for silicon. As the sodium to aluminium ratio increases above 1, more sodium ions are available to screen the aluminosilicate groups. The resulting decrease in their field strength leads to a greater preference for larger cations.

Eisenman (13) has also shown that hydration of the glass has no effect on selectivity order but affects the magnitude of specificity.

Thus, one can conclude that the glass electrode selectivity depends only on anionic field strength and nothing else.

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CHAPTER 4

EXPERIMENTAL WORK

All the chemicals used in this work were analytical grade reagents, except for urea which was laboratory grade reagent and had a purity of 99.0%. The TRIS [tris-(hydroxymethyl)-aminomethane] BUFFER used to maintain a constant pH and a constant ionic strength of the solution was obtained from E.Merck, Germany. The UREASE-ACTIVE MEAL from Jack Beans (each mg. of urease-active meal hydrolyzes 3 mg. urea in 30 minutes at 35°C) used in the enzymatic hydrolysis of urea was obtained from B.D.H., England. Unless otherwise mentioned, four times distilled water was used to prepare the solutions throughout. All ammonium chloride solutions were prepared in 0.1M tris buffer, pH 7.1 \pm 0.1. The method for the preparation of 0.1M tris buffer, pH 7.2 is given in the Appendix.

4-1. PREPARATION OF GLASS.

Two types of glasses with the following compositions were prepared:

- (i) 27 mole-% Na₂O, 3 mole-% Al₂O₃, 67 mole-% SiO₂, and 3 mole-% ZnO (briefly written as NAS 27-3+3 mole-% ZnO).
- (ii) 27 mole-% Na₂O, 4 mole-% Al₂O₃, and 69 mole-% SiO₂ (briefly written as NAS 27-4).

The glass with composition NAS 27-3+3 mole-% ZnO will be referred to as G1, while that having the composition NAS 27-4 will be referred to as G2.

For preparation of the above glasses, the following batch materials were used:

- (i) Sodium carbonate for Na_2O ,
- (ii) Hydrated alumina for Al_2O_3 , and
- (iii) Finely powdered quartz for SiO_2 .

The source of ZnO was analytical grade Zinc Oxide.

Requisite amounts of batch material were weighed to give 100 grams of glass. These were mixed thoroughly in a porcelain mortar and melted in a platinum crucible in an electric furnace for 24 hours at 1450°C . The glasses were cast and thoroughly annealed to get strain free glass slabs.

4-2. PREPARATION OF SILVER-SILVER CHLORIDE INTERNAL ELECTRODES.

A number of silver-silver chloride internal electrodes were prepared by depositing silver chloride electrolytically on silver wire.

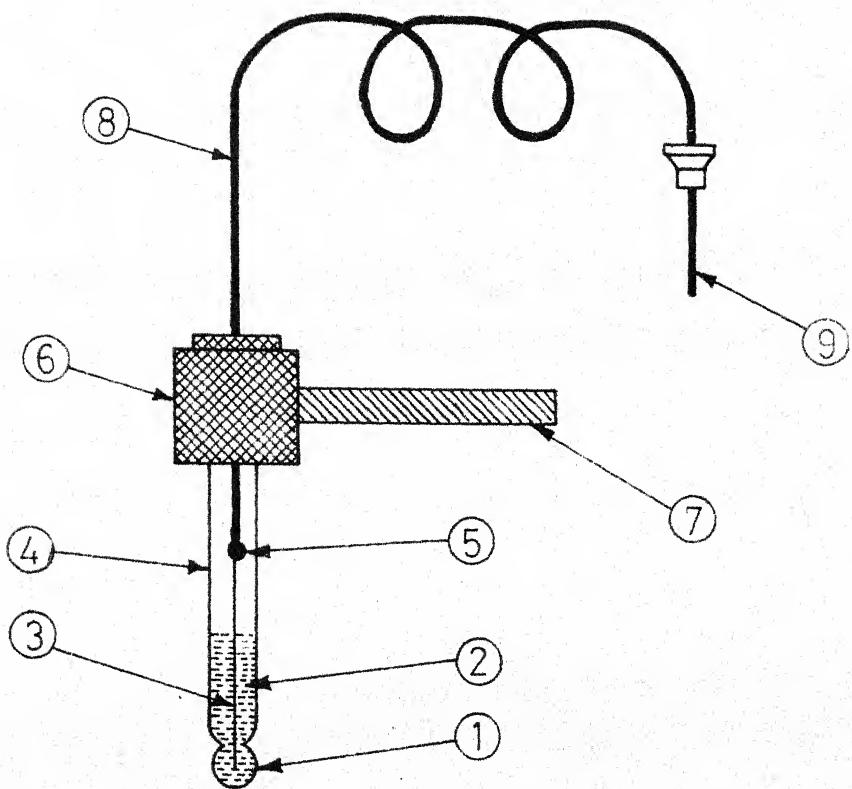
Several silver wires, 33 millimeters in length and 0.8 millimeters in diameter, were cleaned thrice with detergent and then thoroughly washed with multiple rinses of distilled water. The silver wires were then placed in concentrated nitric acid for one hour, following which they were thoroughly washed with multiple rinses of single distilled water, double distilled water, and four times distilled water respectively in a beaker. The wires were then placed on a tissue paper and made air dry without touching them with hand. After this, the silver wires were soldered to hook-up wires, 21 cms. in length.

Silver chloride was then electrolytically deposited on the silver wires one by one. A solution of 0.1N NaCl titrated to pH 11.9 with 3.5 M NaOH was used. 1 ma current was then passed through the electrolytic cell for 30 minutes with the silver wire acting as anode and a platinum wire serving as cathode. During the process there was a slow deposition of a fine coat of grey silver chloride on the wire. Finally all the silver - silver chloride internal electrodes were stored in 0.1N NaCl solution, pH 11.9.

4-3. FABRICATION OF ELECTRODES.

The principles of construction of the conventional bulb-type glass electrode are shown in Figure 2.

Five ammonium ion-sensitive glass electrodes of the bulb-type were fabricated—three from the glass G1 and two from the glass G2. In one of the electrodes constructed from the glass G1, a platinum wire of diameter 0.2 mm was used as the internal reference electrode; while in the remaining two, silver-silver chloride was used. 0.1N NaCl, buffered to pH 7.42 with tris was used as the internal filling solution for the electrode prepared from the glass G1 and having platinum wire as the internal reference electrode. Out of the remaining two electrodes fabricated from the glass G1, one contained 0.2M NH_4Cl solution having pH 7.29 as the internal filling solution, while the other contained 0.1N NaCl buffered to pH 7.27 with tris. Silver-silver chloride was used as the internal reference electrode for



- 1 - Bulb-shaped ion-selective glass membrane.
- 2 - Buffered internal filling solution.
- 3 - Internal reference electrode (e.g. Ag-AgCl)
- 4 - Glass stem.
- 5 - Solder joint.
- 6 - Cap.
- 7 - Ebonite rod for mounting the electrode in the holder
- 8 - Electrode lead.
- 9 - Terminal connector.

Fig. 2 - Constructional features of a bulb-type glass electrode.

the two electrodes constructed from the glass G2 but the internal filling solution was different. For one of them 0.2M NH₄Cl solution having pH 7.29 was used and for the other, 0.1N NaCl buffered to pH 7.27 with tris. The electrodes were numbered accordingly and they are summarized in Table 1.

The detailed method of fabrication of a typical bulb-type glass electrode sensitive to ammonium ions is described below:

1. A search was made to find out a suitable stem glass to which the glasses G1 and G2 could fuse. These were found to fuse to lead glass.
2. Therefore, a lead glass tubing of 4 mm I.D. and 1 mm wall thickness was drawn by first heating the available tubing of higher diameter to its melting point in a controlled Indane gas-air flame to avoid blackening of the lead glass and then stretching it. The portion having an uniform shape and diameter was cut to length from the tubing thus prepared.
3. The lead glass tubing having 4 mm. I.D. was heated near the centre, and drawn slightly apart, forming a narrow neck. The tube was cut in the centre of the narrow area.
4. The narrow end of one of the tubes was heated to the melting point in a controlled Indane gas-air flame.
5. Simultaneously, a small pea-sized fragment of the ammonium ion-sensitive glass was heated and brought into contact with the stem glass tube and allowed to fuse.

TABLE 1: TYPES OF AMMONIUM ION-SENSITIVE GLASS ELECTRODES FABRICATED

Electrode No.	Type of the Glass Used for Bulb	Internal Reference Electrode	Internal Filling Solution
1	G1	Platinum wire	0.1N NaCl, pH 7.42
2	G1	Ag -AgCl	0.2M NH ₄ Cl, pH 7.29
3	G1	Ag -AgCl	0.1N NaCl, pH 7.27
4	G2	Ag -AgCl	0.2M NH ₄ Cl, pH 7.29
5	G2	Ag -AgCl	0.1N NaCl, pH 7.27

6. By blowing into the opposite end of the tube of stem glass, while continuously keeping the ammonium ion-sensitive glass in molten state, a thin-walled bubble approximately 5 to 6 mm in diameter was formed.
7. The stem-bulb assembly was allowed to cool, following which the electrode measuring approximately 4.5 cms. in length from the bottom of the bulb was cut.
8. The inside of the electrode was filled to half of its length with buffered internal filling solution using a 2-cc hypodermic syringe.
9. The silver-silver chloride electrode was inserted deep into the stem as shown in Figure 2 and the other end of the hook-up wire serving as the electrode lead was passed through a cap made out of nylon rod.
10. The stem was fixed into the cap with Araldite adhesive and all possible openings in the cap were sealed to avoid any leakage with the same adhesive.
11. Finally, a terminal connector was soldered to the electrode lead as shown in Figure 2.

After the fabrication of the electrodes had been completed, they were stored in five separate beakers containing four times distilled water for the preconditioning of the outer surface of the ammonium ion-sensitive glass membrane.

4-4 PROCEDURE FOR MEASURING THE RESPONSE OF ELECTRODES TO AMMONIUM IONS.

All experiments were carried out in a small air conditioned room whose temperature was held constant at $28.0 \pm 1.0^\circ\text{C}$. All potentiometric measurements were made using a Beckman Model G pH meter. A fibre type saturated calomel electrode (S.C.E.) obtained from the Central Glass and Ceramic Research Institute, Calcutta served as the external reference electrode. Ammonium chloride solutions of constant pH and ionic strength were prepared fresh for every run. The pH of the ammonium chloride solutions was maintained above 7.0 to minimize any interference from H^+ -ions. To measure the e.m.f. of the cell as a function of time, a stop-watch was employed.

The procedure used to measure the response of electrodes to ammonium ions is as follows:

1. 25.0 ml of 1.0M standard ammonium chloride solution was prepared in 0.1M tris buffer, pH 7.1 ± 0.1 .
2. Then a series of 25.0 ml of standard ammonium chloride solutions ranging in concentration from 1.0×10^{-1} to $1.0 \times 10^{-4}\text{M}$ (1.0×10^{-1} , 1.0×10^{-2} , 1.0×10^{-3} , and $1.0 \times 10^{-4}\text{M}$) were prepared by successive dilution with 0.1M tris buffer, pH 7.1 ± 0.1 and their pH noted.
3. The ammonium ion-sensitive glass electrode and the saturated calomel electrode were connected to the respective terminals of the Beckman Model G pH meter. Both of them were rinsed with four times distilled water and gently wiped dry with an

absorbent tissue paper.

4. 7.0 ml of the required ammonium chloride solution was pipetted into the sample beaker and placed in the holder.
5. The holder was raised into the position so that electrode tips were in sample.
6. As soon as the electrode tips touched the sample, the stopwatch was started and the sample compartment door closed.
7. Millivolt readings were then noted at every five minutes interval for the first 30 minutes, at every 10 minutes interval for the next 60 minutes and thereafter at every 30 minutes interval till the steady-state e.m.f. was attained. The first reading, however, was taken after one minute from the time of immersion.

Unless otherwise mentioned, all e.m.f. measurements with ammonium chloride solutions were made in the decreasing order of NH_4^+ concentration. Three rinses with four times distilled water were interposed when changing solutions from higher concentration to lower concentration. After each rinsing, the bulbs of the electrodes were gently touched with an absorbent tissue paper to remove adhering liquid. At each experimental condition, duplicate experiments were performed in order to check their reproducibility.

4-5. PROCEDURE FOR DETERMINATION OF UREA.

The potentiometric method used for measuring the activity of ammonium ions produced during the urease catalyzed hydrolysis

of urea with the help of ammonium ion-sensitive glass electrodes fabricated in our laboratory is described below.

The following stock solutions were prepared and remade for each experiment:

Urea Solution: A 500 ml solution containing 0.7508 gram of urea was prepared in four times distilled water.

Urease Solution: 1.0 gram of urease-active meal was extracted with 100 ml of four times distilled water for 1 hour with frequent stirring and the insoluble residue allowed to settle down. The extract was then filtered and filtrate stored in a stoppered 100-cc volumetric flask at room temperature.

Aliquots containing 37.54 and 3.754 mg of urea were pipetted into two 250-cc volumetric flasks referred to as VF-1 and VF-2 respectively. 10 ml of the urease solution was then added to VF-1 and 5 ml to VF-2. 100 ml of four times distilled water was added to each flask. The flasks were stoppered and placed in the Aquatherm Water Bath Shaker (bath temperature = $35.0 \pm 0.1^{\circ}\text{C}$ and shaker speed = 150 RPM), time noted and reaction allowed to proceed. Exactly after 45 minutes, the flasks were removed from the bath and allowed to stand for 30 minutes at room temperature. The contents of each flask were then diluted to the mark with 0.1M tris buffer, pH 7.1 ± 0.1 and their pH measured. The procedure described in section 4-4 was followed for the potentiometric measurements, except that in this case the sample solutions were the contents from the flasks VF-1 and VF-2.

instead of ammonium chloride solutions. First, the e.m.f. was measured for the solution from the flask VF-1, and then for the solution from the flask VF-2. Urea concentrations were determined from the calibration curves prepared earlier. The electrode no.1 had to be recalibrated because more than half of its internal filling solution had evaporated changing the activity of the inner solution.

4-6. RESPONSE OF ELECTRODES SOAKED IN 0.1M NH₄Cl, pH 7.1.

To study the effect of soaking the electrodes in NH₄⁺ - solution on their response characteristics, the electrode numbers 2 to 5 were placed in four separate beakers containing 0.1M NH₄Cl solution, pH 7.1. The period of soaking varied from 24 hours to 82.5 hours for various electrodes. The ammonium ion concentrations for which the electrode responses were measured were 1.0×10^{-1} M and 1.0×10^{-2} M. Potentiometric measurements were made by the method, as explained earlier, but in the increasing order of NH₄⁺ concentration. During the course of e.m.f. measurements, no distilled water rinses were used when changing the sample solution from lower concentration to higher concentration.

Finally, at the end of these experiments, all the electrodes were placed back in four times distilled water, and after sufficient period of soaking, they were tested for any deviation from their original response to ammonium ions.

CHAPTER 5

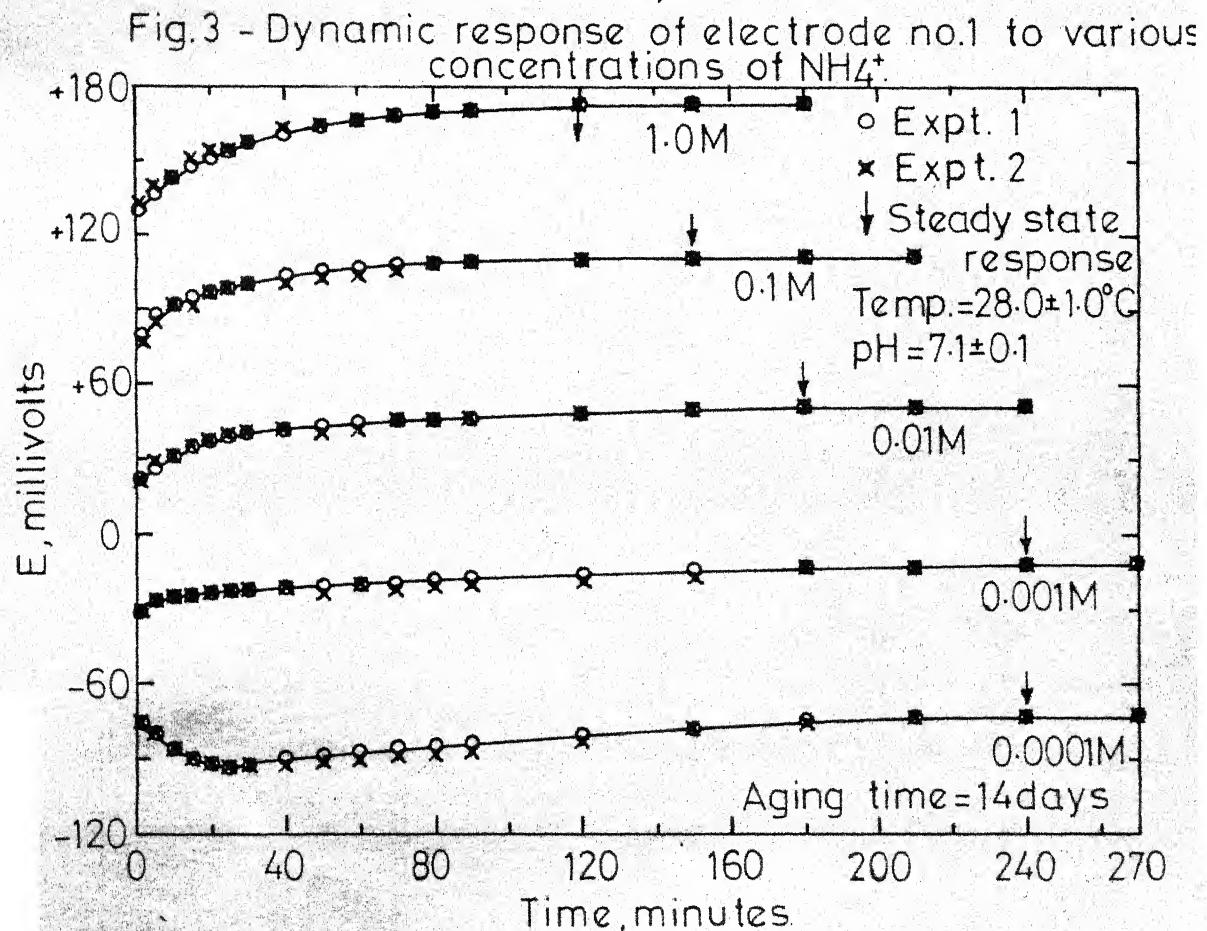
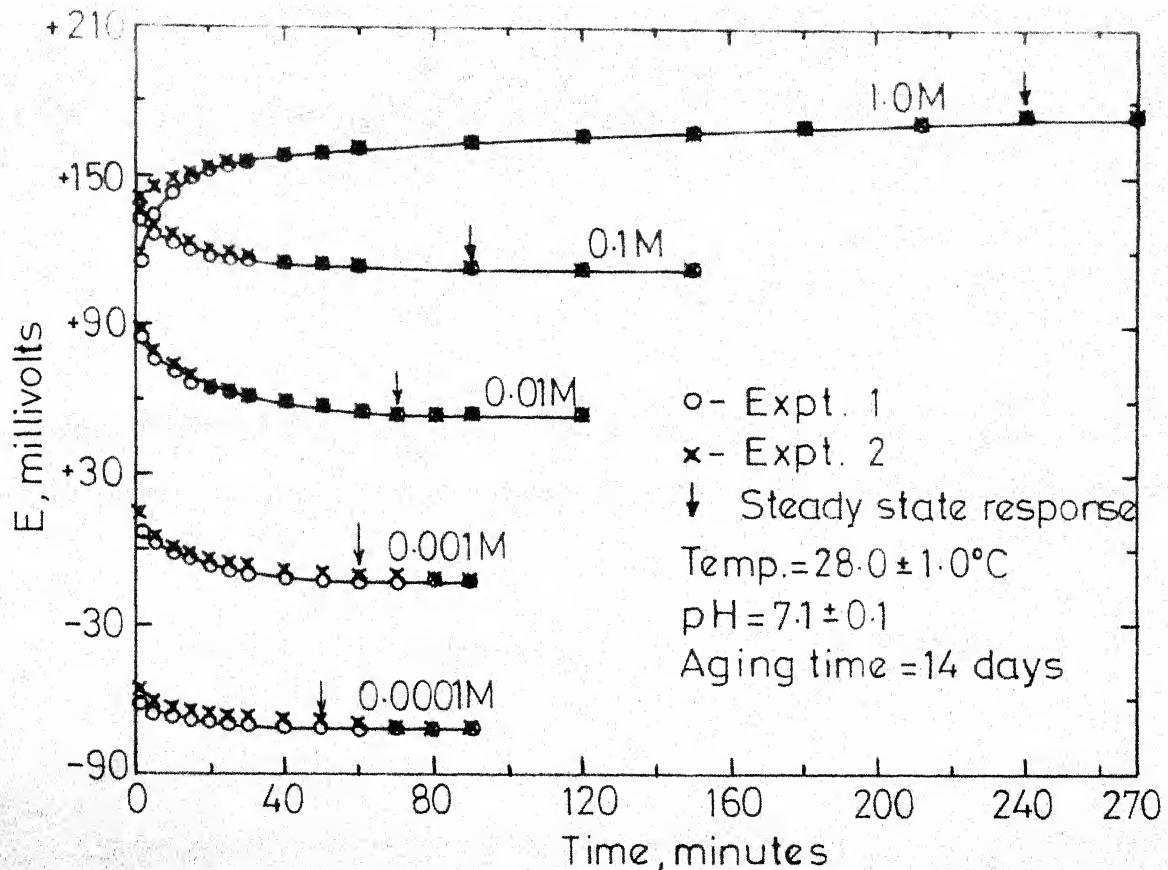
RESULTS AND DISCUSSION

The results of the present studies of response of the ammonium ion-sensitive glass electrodes towards NH_4^+ as well as their application in urea determination are presented and discussed below.

5-1. DYNAMIC RESPONSE OF THE ELECTRODES.

The electrodes were removed from the beakers after an ageing period of 14-30 days in four times distilled water and tested for their response to NH_4^+ . The response curves obtained for electrode numbers 1 to 5 in ammonium chloride solutions of various concentrations are shown in Figures 3, 5, 6, 7 and 8 respectively, where E_{cell} has been plotted as E against time after immersion of the electrodes in each new solution. The sequence of e.m.f. measurements was from more to less concentrated solutions.

It is seen that the response curves for all the electrodes are smooth and of nearly identical shape. An excellent reproducibility of the steady-state e.m.f. values is observed in all the cases. All the electrodes are found to have e.m.f. drifts in the positive direction for 1.0M NH_4Cl ; but for other concentrations used in this work, they are in the negative direction.



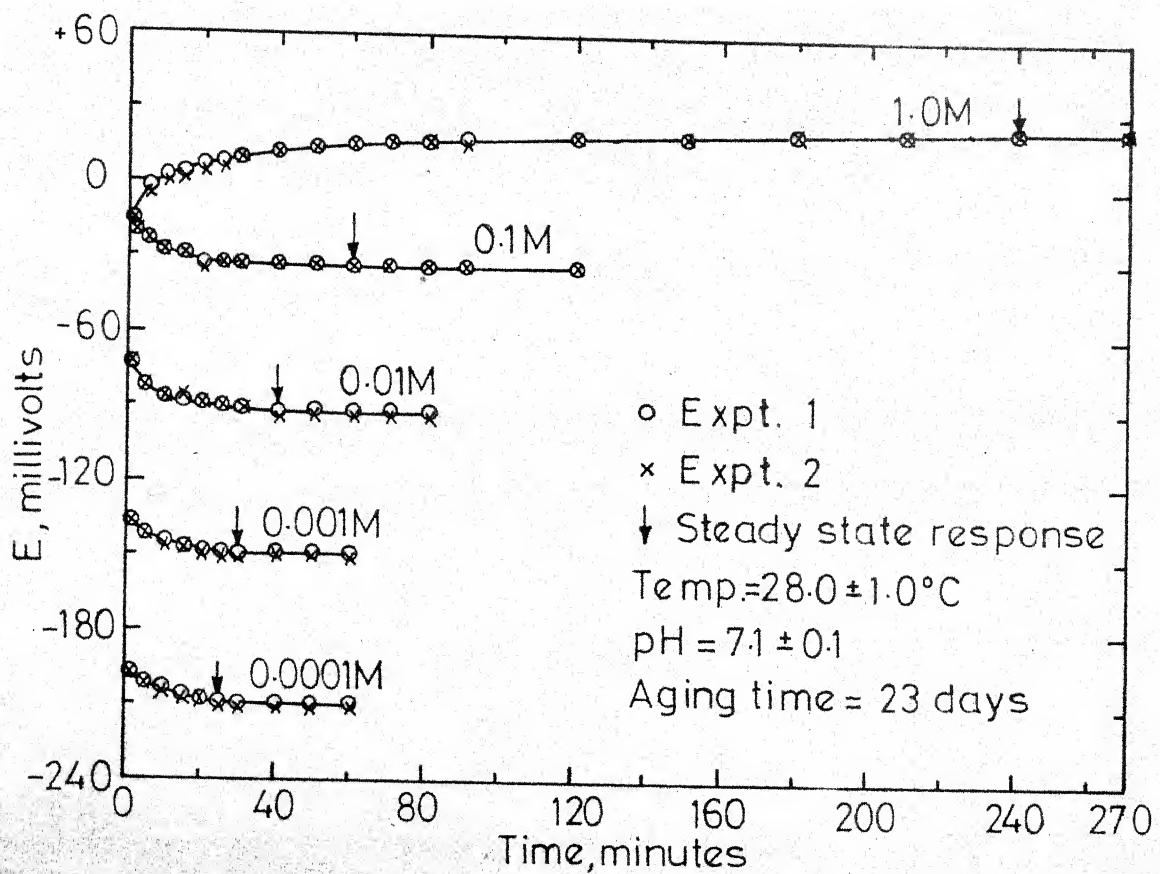


Fig. 5 - Dynamic response of electrode no. 2 to various concentrations of NH_4^+ .

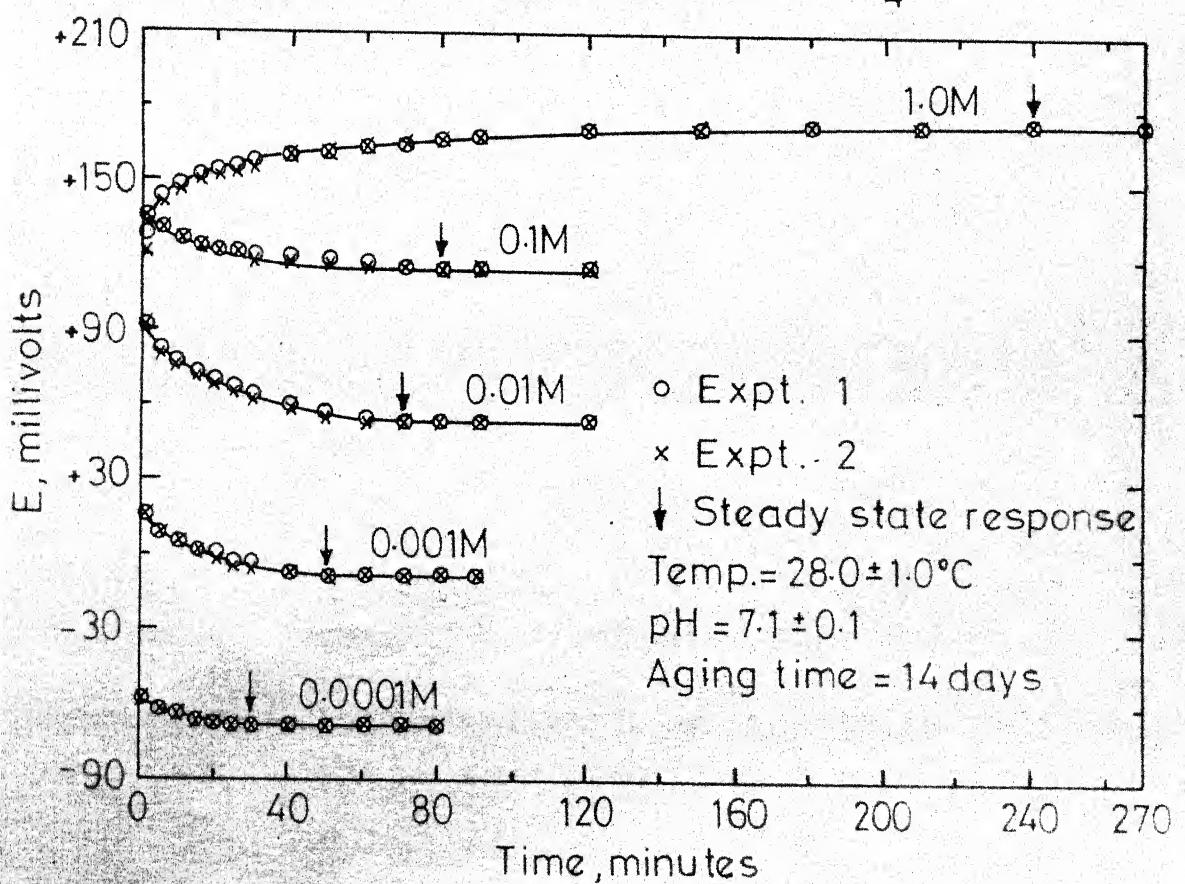


Fig. 6 - Dynamic response of electrode no. 3 to various concentrations of NH_4^+ .

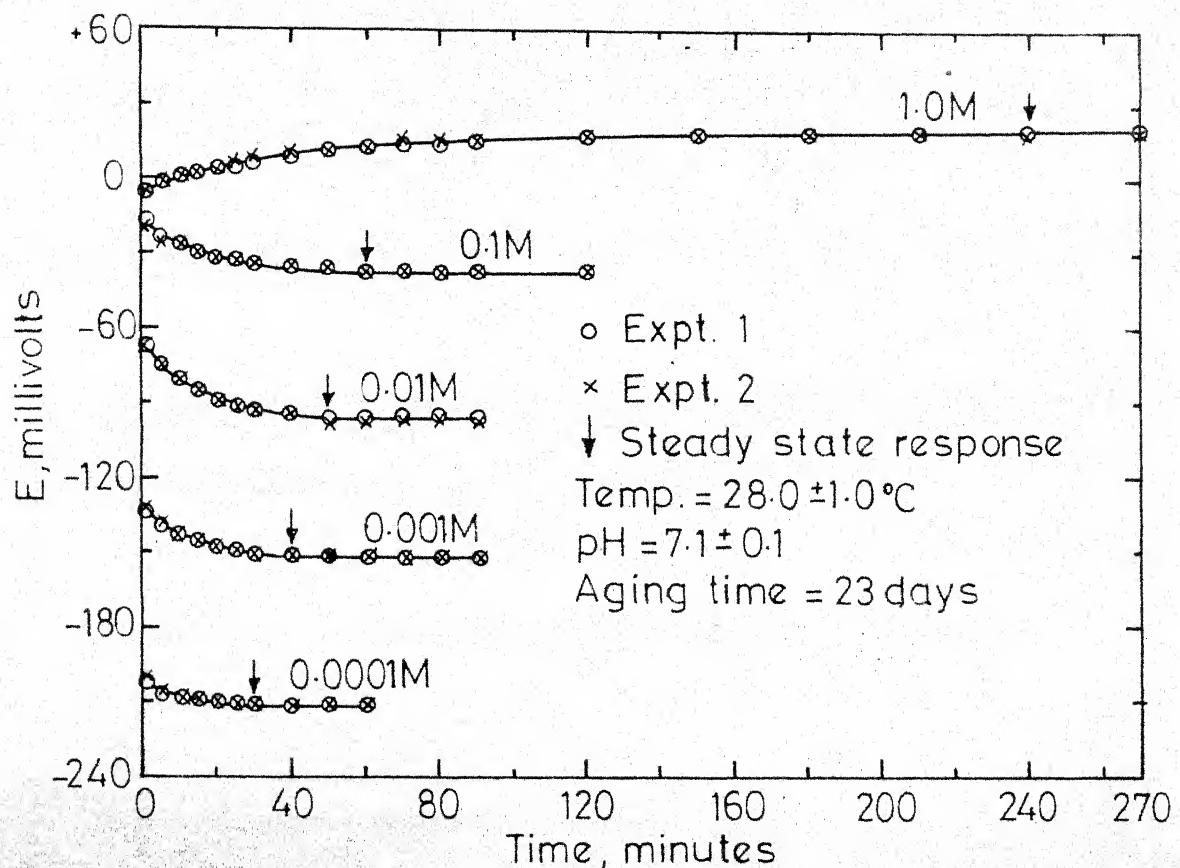


Fig. 7 - Dynamic response of electrode no.4 to various concentrations of NH_4^+ .

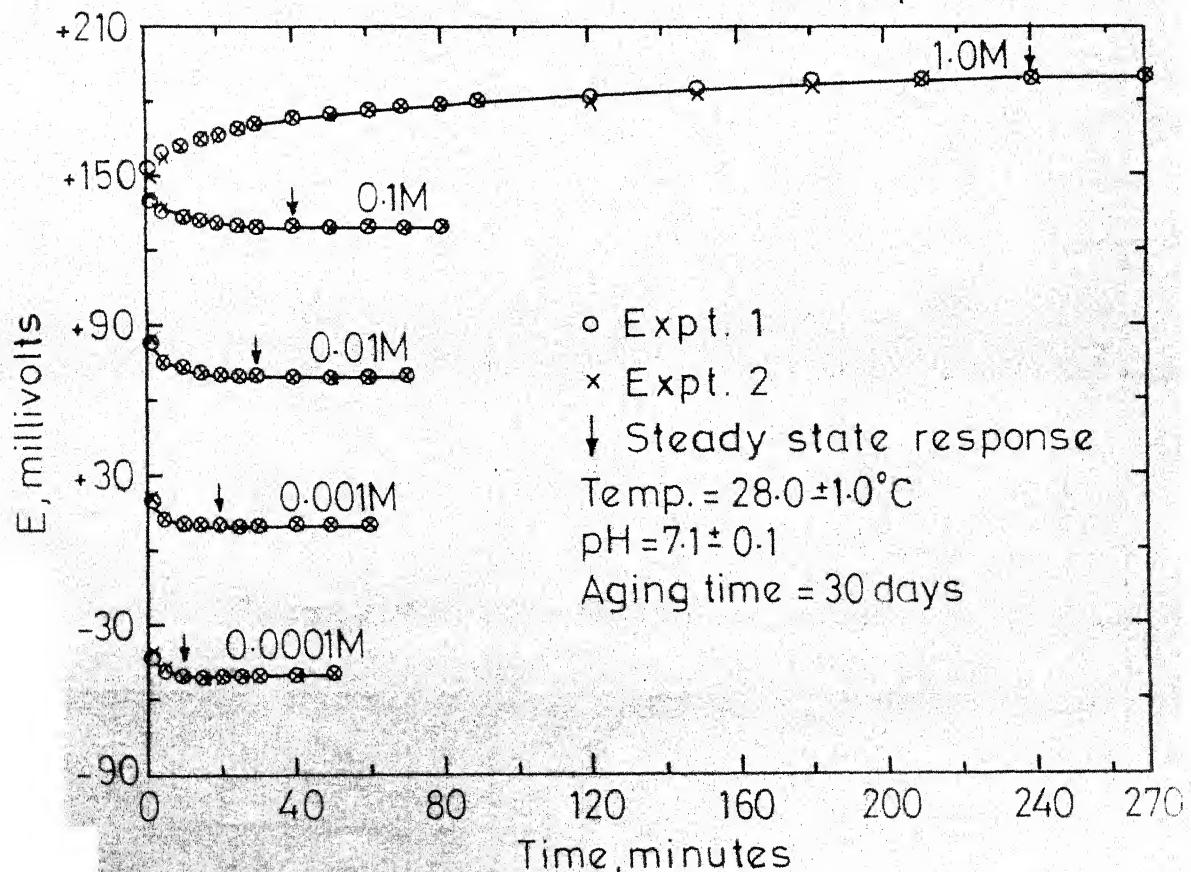


Fig. 8 - Dynamic response of electrode no.5 to various concentrations of NH_4^+ .

This can be explained on the basis of ion-exchange taking place between external hydrated layer of glass and solutions. When the glass electrode, soaked in four times distilled water, comes into contact with the test solution, rapid ion-exchange takes place on the glass surface, and the sorbed ions then diffuse into the depth of the glass. As the ion-exchange proceeds with time, the e.m.f. keeps rising until the ion-exchange equilibrium is established at the gel layer - solution interface. The number of ammonium ions available for ion - exchange becomes smaller when the ammonium ion concentration is lowered. Therefore, when an electrode, initially at steady-state in 1.0M NH_4Cl is placed in a solution of lower ammonium ion concentration (after rinsing it thrice in four times distilled water and then wiping out the adhering liquid with an absorbent tissue paper), the electrode potential is thought to be higher than the steady-state potential of the electrode in the solution it is being tested in, and hence, the e.m.f. is considered to be decreasing with time until the equilibrium value is attained. The response curves show that the magnitude of the e.m.f. drift is maximum for 1.0M NH_4^+ and minimum for 0.0001 M NH_4^+ .

The response rates of all the electrodes are observed to be extremely slow. The slow response may be due to a high wall thickness of the bulb which is of the order of 0.5 mm and due to low degree of hydration of the glass, poor surface characteristics, and non-uniform chemical properties of the hydrated layer of the

glass. Mattock and Uncles (26) and Savage and Isard (66) have reported that washing the electrodes with distilled water and wiping with a filter paper between tests may cause sluggish response.

The nature of the response curves are found to be nearly similar for both the glasses, G1 and G2. Internal filling solution and internal reference electrode are seen to have no effect on the nature of the response curves.

A. Effect of Ammonium Ion Concentration on the Response Rate:

It is found from the experimental curves that the time taken for the electrodes to register a stable potential in 1.0M NH_4Cl is 4 hours in all the cases; but with decrease in ammonium ion concentration, the response is observed to be comparatively faster. Slow equilibration of the surface concentrations, when the electrodes are transferred to 1.0 M NH_4Cl from four times distilled water, is thought to be responsible for a high response time of 4 hours. It has already been seen that when a soaked glass electrode is brought into contact with the solution, rapid ion-exchange takes place and the sorbed ions then diffuse into the glass. Since the ion-exchange is thought to be instantaneous, it is probably the slow rate of diffusion that is causing the slow equilibration of the surface concentrations and hence, a very slow response to 1.0M NH_4Cl is seen. When the ammonium chloride solutions become more and more dilute, the number of

ammonium ions available for ion-exchange and subsequently for diffusion becomes smaller and smaller, and possibly the ion-exchange is limited only to the glass surface in the case of extremely dilute solutions. Therefore, the response time decreases with decrease in ammonium ion concentration.

B. Effect of Glass Composition on the Response Time:

The time dependent curves of Figures 5 and 7 show that the response times of the electrode numbers 2 and 4, aged for 23 days in four times distilled water before use, are same when tested in 1.0 and 0.1M NH_4Cl . However, the response of the glass G1 is found to be relatively faster by 10 minutes in 0.01 and 0.001 M NH_4^+ , and by 5 minutes in 0.0001 M NH_4^+ over the glass G2. These differences of 5 and 10 minutes in the response times are not of much significance when compared to the times of 30-60 minutes required to attain the stable e.m.f. in ammonium chloride solutions of various concentrations. On the other hand, it is seen from Figures 6 and 8 that the response of the glass G2 is relatively much faster than the glass G1 except in 1.0M NH_4Cl in which the response times are the same for both the glasses, G1 and G2. Therefore, it is suggested that these differences in the response times may not be due to difference in glass but in ageing time which is 14 days for the electrode number 3 and 30 days for the electrode number 5. However, other investigators (65-67) have reported the variation of response time with glass composition.

C. Effect of Internal Filling Solution on the Response Time:

A comparison of Figures 5 and 6 shows that the response time in 1.0M NH_4Cl is same for the electrode numbers 2 and 3 containing 0.2M NH_4Cl and 0.1N NaCl as the internal filling solution respectively. On passing from more to less concentrated solutions, however, the electrode number 2 is found to have relatively faster response over the electrode number 3. On the contrary, when the experimental curves of Figures 7 and 8 are compared, the electrode number 5 containing 0.1N NaCl as the internal filling solution is seen to have relatively faster response than the electrode number 4 containing 0.2M NH_4Cl as the inner filling solution in all the external concentrations used except 1.0M in which the response time is the same for two electrodes. On the basis of these results, it is, therefore, difficult to predict any effect of internal filling solution on the response time since ageing time is not same for all these electrodes. These contrarieties of response times are thought to be due to entirely different surface characteristics of the electrodes which may result from difference in structure, in the degree of hydration, and in the chemical properties of the gel layer of the glass.

D. Effect of Internal Reference Electrode on the Response Time:

The experimental curves of Figures 3 and 6 indicate that the response times of the electrode numbers 1 and 3 having platinum and silver-silver chloride as the inner reference electrode

respectively are same in 1.0 and 0.01M NH_4Cl but different in the other concentrations used. It is found that the difference in the response times of the two electrodes in 0.1 and 0.001 M NH_4^+ is only 10 minutes which may be considered to be insignificant compared to the times taken to register equilibrium e.m.f. in ammonium chloride solutions of various concentrations. However, in the case of 0.0001M NH_4Cl , there is a difference of 20 minutes in the response times of the two electrodes, although the ageing time is same. This single significant difference cannot form the basis of claiming any dependence of response time on the internal reference electrode. However, because of the structural and hydration effects at the glass surfaces, the observed differences in the response times of the two electrodes can arise.

E. Effect of Direction of the Concentration Jump on Response Characteristics:

To study the effect of direction of the concentration jump on the nature of the response curves and on response time, the electrode number 1 was tested in the sequence of less to more concentrated solutions of ammonium chloride and the results are shown in Figure 4. The direction of the e.m.f. drift is found to be reversed for all the concentrations of NH_4^+ except 1.0M, when the e.m.f. measurements are done in the sequence of less to more concentrated solutions instead of more to less concentrated solutions. The response curves are seen to have 'normal' e.m.f. drifts in all the cases except for 0.0001M NH_4Cl .

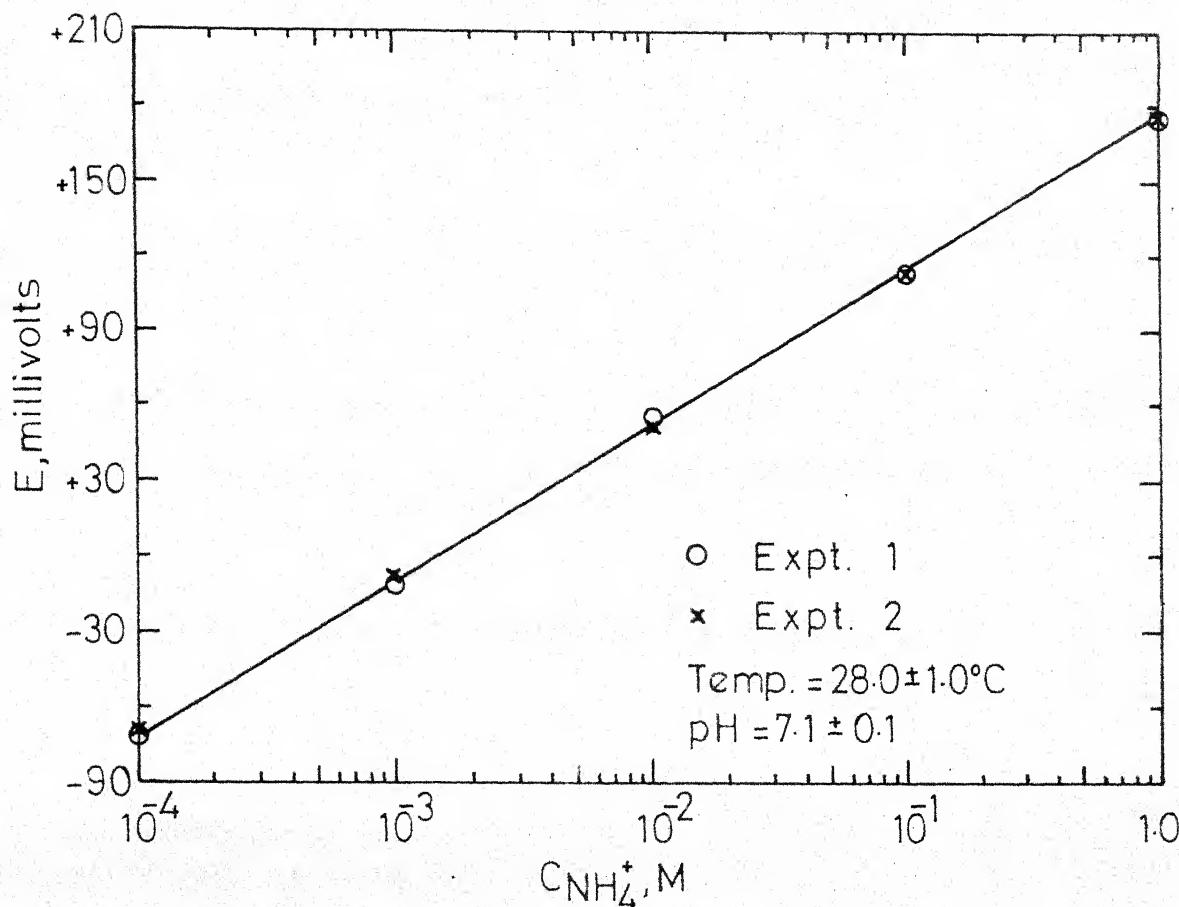


Fig. 9 -Nernst response of electrode no.1 to NH_4^+ .

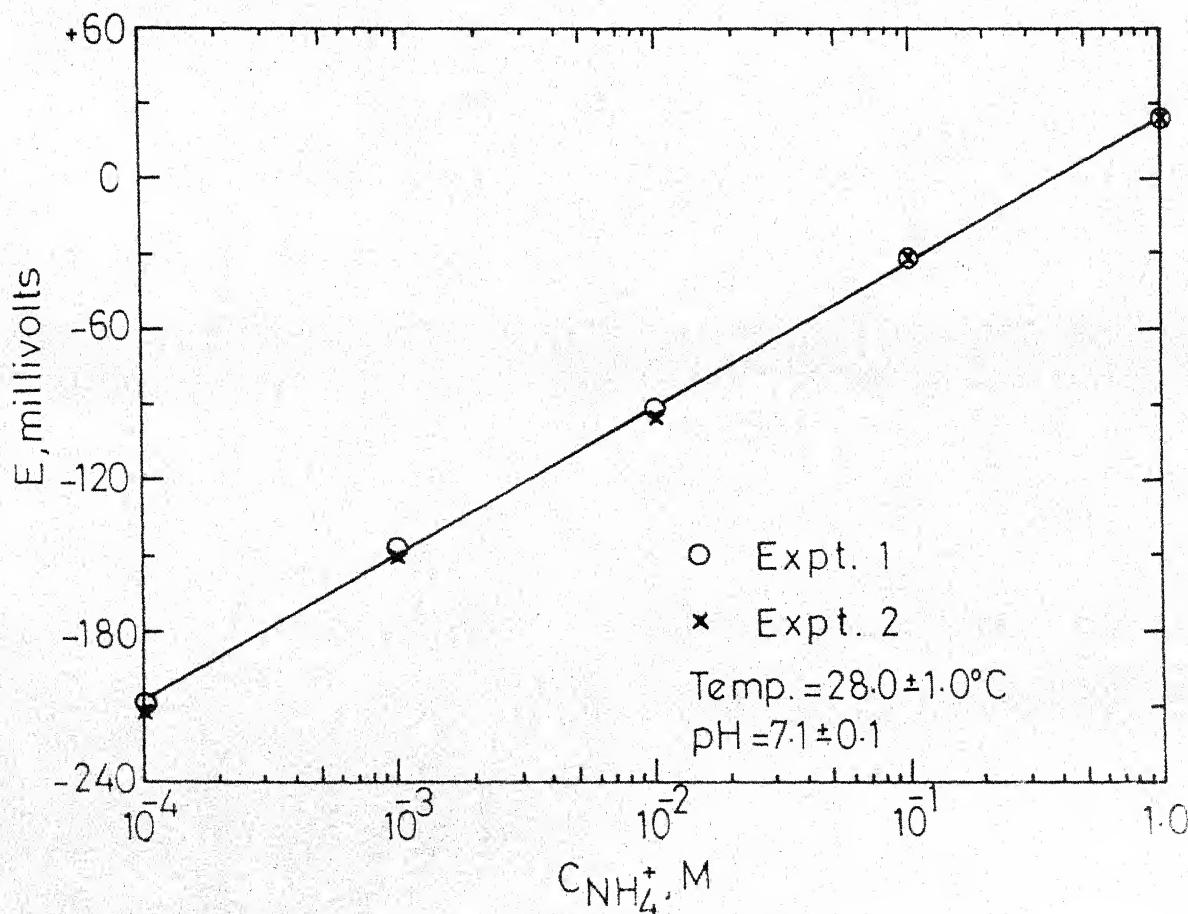


Fig.10-Nernst response of electrode no.2 to NH_4^+ .

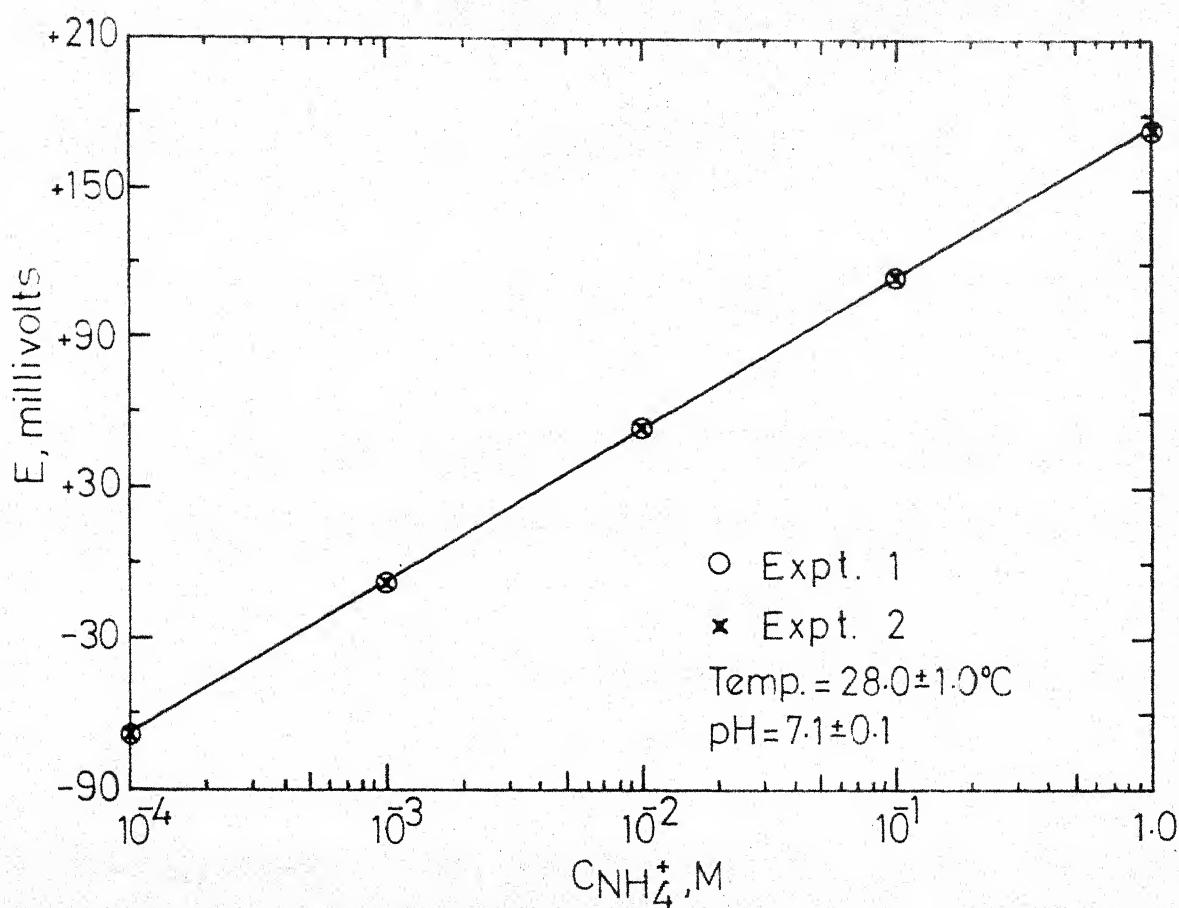


Fig.11 - Nernst response of electrode no.3 to NH_4^+ .

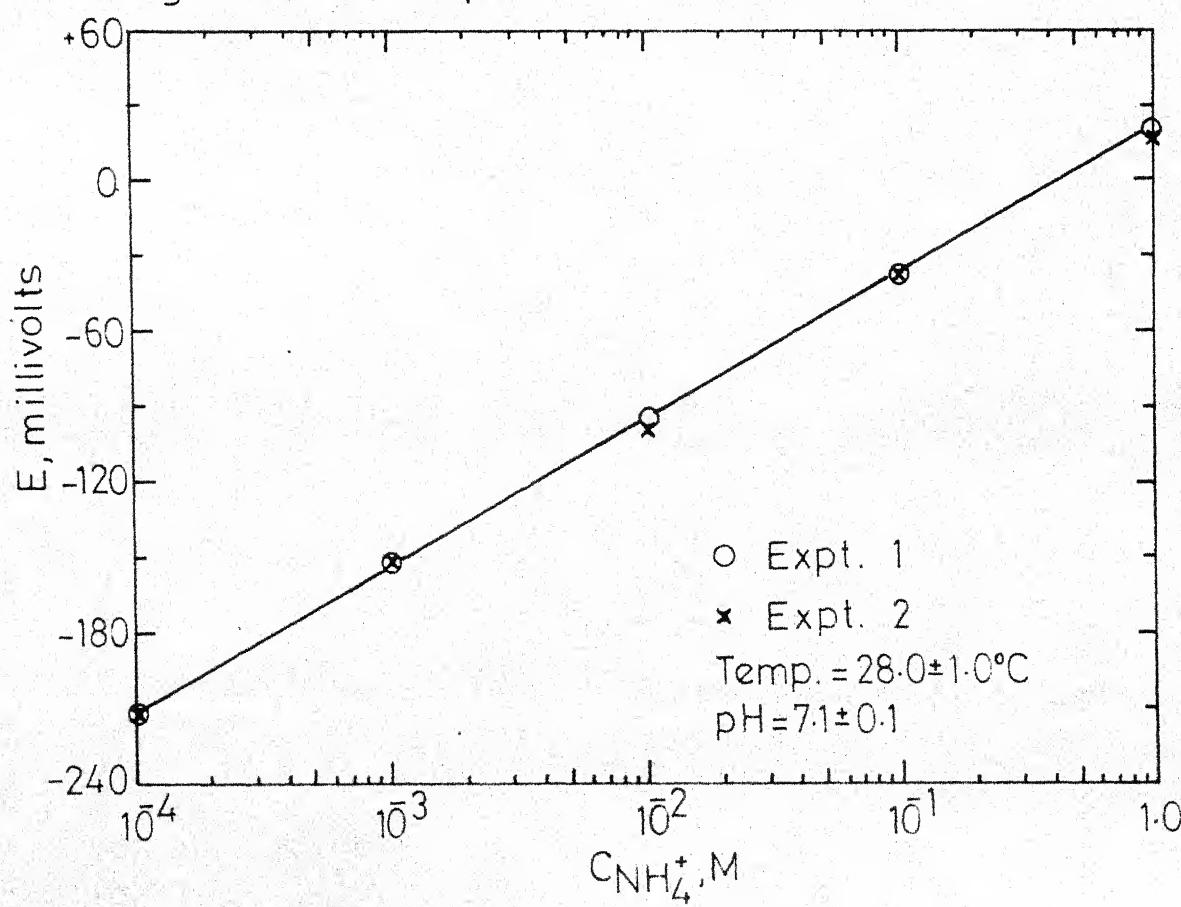


Fig.12 - Nernst response of electrode no.4 to NH_4^+ .

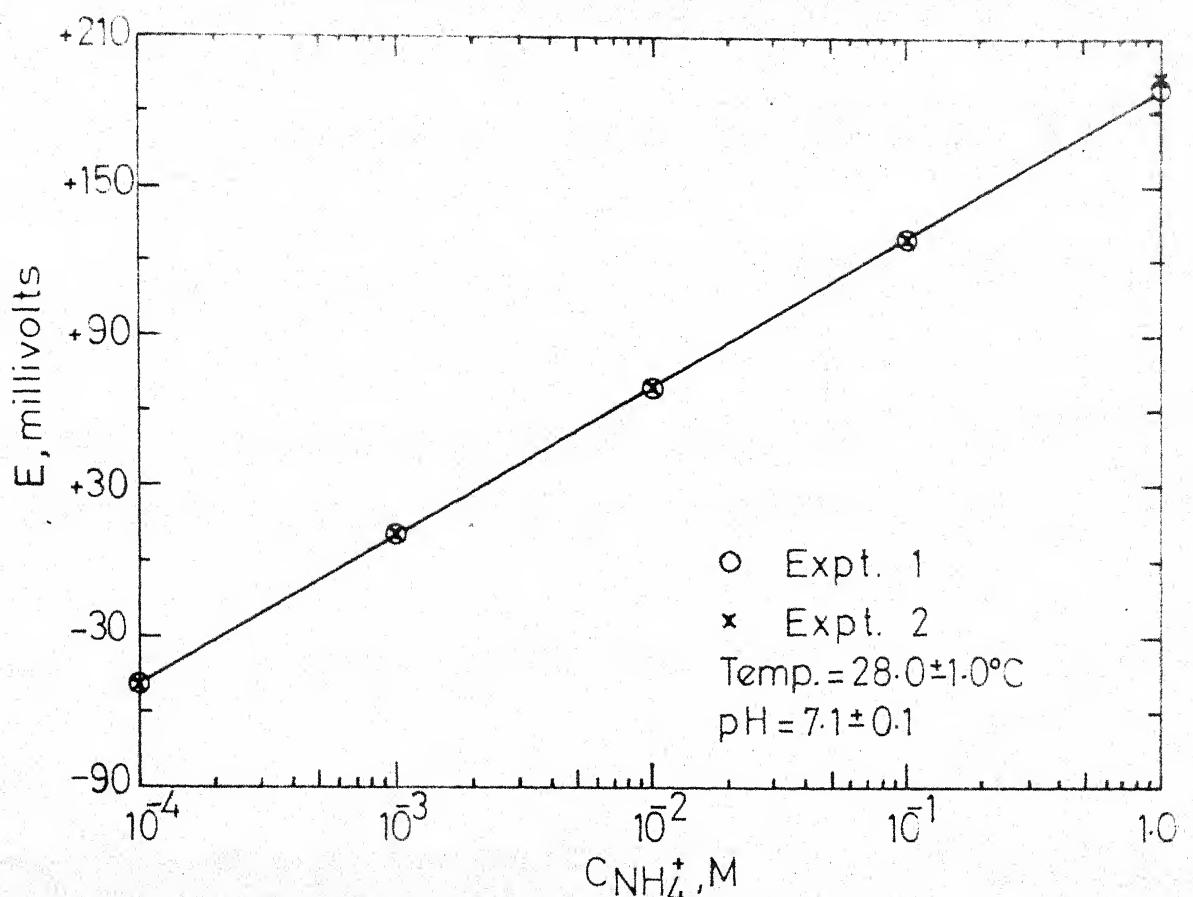


Fig.13-Nernst response of electrode no. 5 to NH_4^+ .

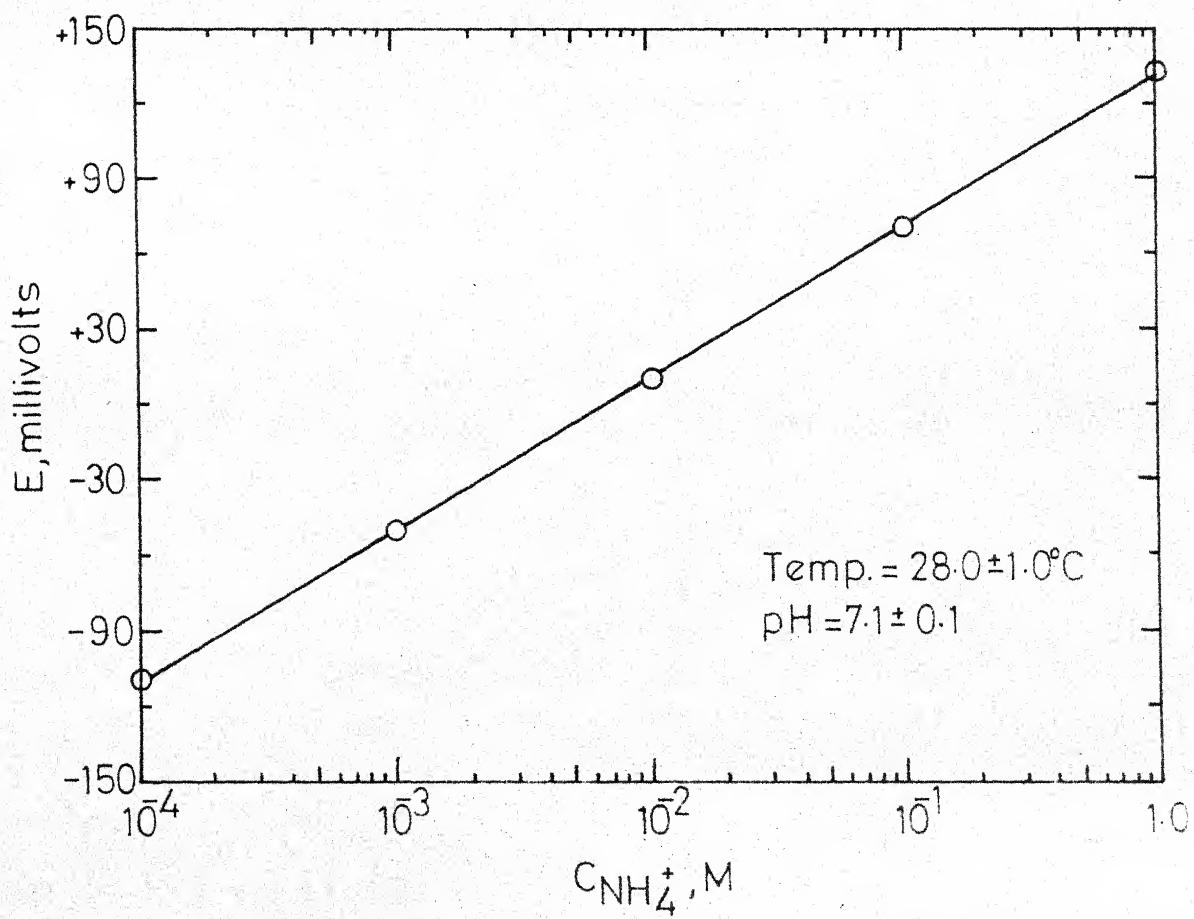


Fig.14-Calibration plot for the determination of urea by use of electrode no. 1.

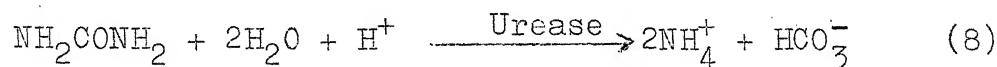
for which the e.m.f. first decreases with time and then increases. In the present studies, no attempt has been made to explain the nature of the initial part of the experimental response curve for 0.0001M NH₄Cl.

5-2. NERNST RESPONSE OF THE ELECTRODES:

For a glass electrode to be responsive to ammonium ions, a plot of E versus logarithm of NH₄⁺ concentration should be a straight line with a slope of 59.7 mV at 28°C according to the Nernst equation. The results of such plots for electrode numbers 1 to 5 are shown in Figures 9, 10, 11, 12 and 13 respectively, where the steady state response of the electrodes to NH₄⁺ in the concentration range 1.0 to 0.0001 M has been plotted as E on a linear ordinate scale against the ammonium ion concentration, C_{NH₄⁺} on a logarithmic abscissa scale. All the electrodes are found to give completely Nernstian response to ammonium ions with excellent reproducible voltages. The theoretical and experimental values of the Nernst slope for various electrodes at 28°C are summarized in Table 2 and the relative error is found to be less than 5.2%.

5-3. DETERMINATION OF UREA.

The ammonium ion-sensitive glass electrodes can be used for the determination of urea via complete conversion of urea to NH₄⁺ by enzyme-catalyzed hydrolysis:



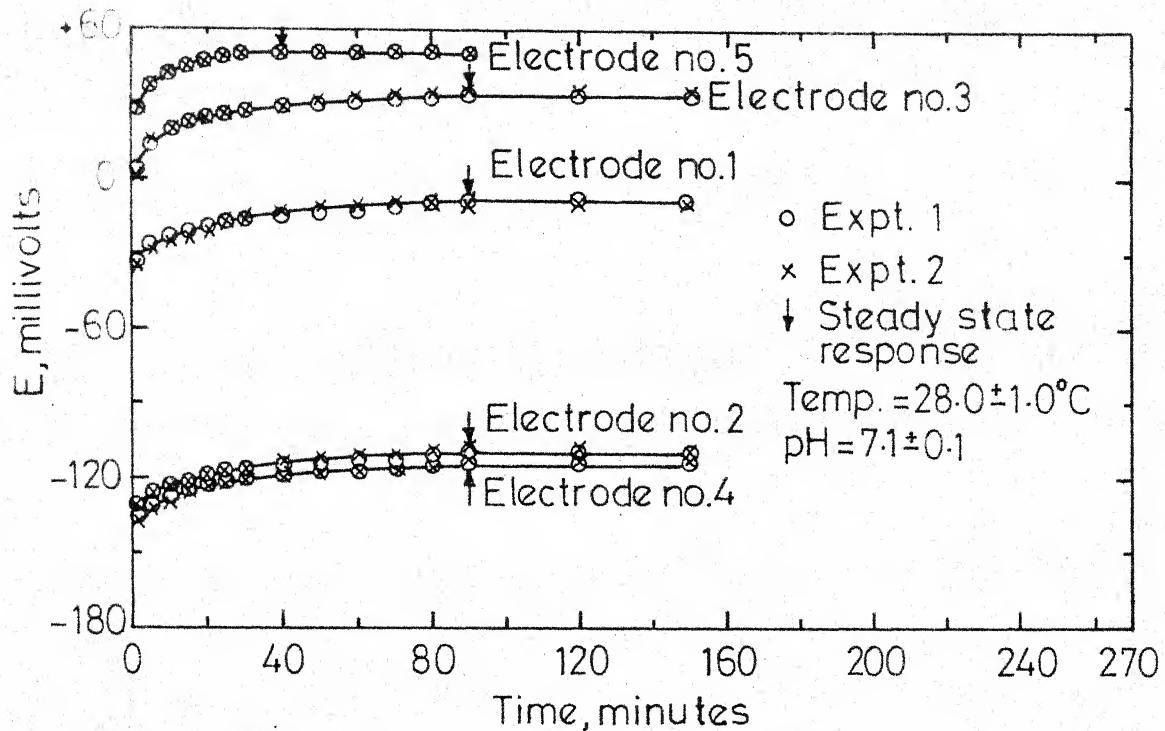


Fig.15-Emf vs. time curves obtained with various electrodes for 0.619 millimoles of urea hydrolyzed.

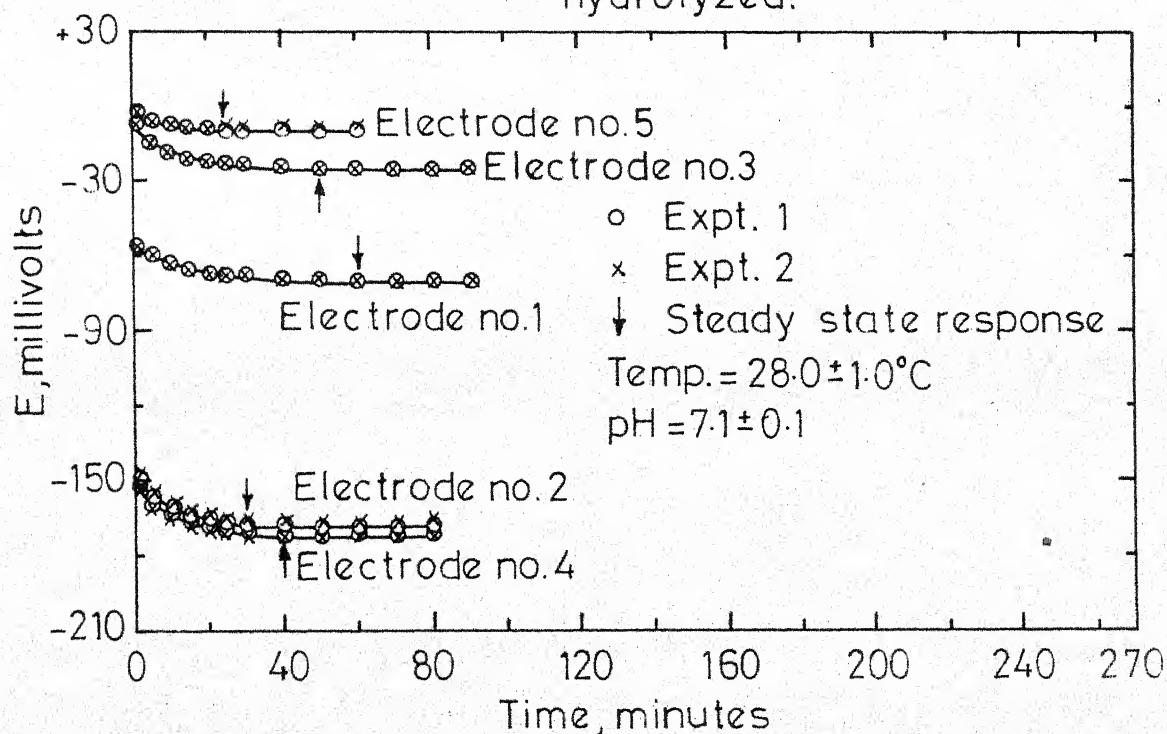


Fig.16-Emf vs. time curves obtained with various electrodes for 0.0619 millimoles of urea hydrolyzed.

TABLE 2: NERNST SLOPE FOR VARIOUS ELECTRODES AT 28°C

Electrode No.	Theoretical	Nernst Slope, mV	Relative Error, %
		Experimental	
1	59.7	62.8	5.19
2	59.7	58.3	2.34
3	59.7	61.0	2.18
4	59.7	58.5	2.01
5	59.7	60.0	0.50

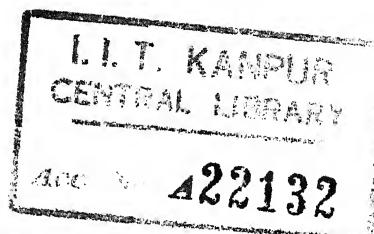
These ammonium ions are sensed by the electrode and the steady-state e.m.f. developed is proportional to the amount of NH_4^+ produced and hence to the concentration of urea.

The millivolt readings obtained with various electrodes for 0.619 and 0.0619 millimoles of urea hydrolyzed are shown in Figures 15 and 16 respectively as a function of time. The nature of the curves are found to be similar to those for ammonium chloride solutions. The response rates are found to be faster in these cases compared to ammonium chloride solutions, although the ageing time is nearly same. The reasons are not clear.

In order to find the error involved in determining the concentration of urea with the help of ammonium ion-sensitive glass electrodes, the steady-state millivolt readings are converted into NH_4^+ concentration values by using the curves shown in Figures 10, 11, 12 and 13 as calibration curves for the

TABLE 3: RESULTS OF UREA DETERMINATIONS

Electrode No.	Urea taken, millimoles	Urea found, millimoles	Relative Error, %
1	0.619	0.613	0.97
		0.588	5.00
	0.0619	0.0600	3.07
		0.0600	3.07
2	0.619	0.600	3.07
		0.600	3.07
	0.0619	0.0588	5.00
		0.0600	3.07
3	0.619	0.588	5.00
		0.600	3.07
	0.0619	0.0625	0.97
		0.0625	0.97
4	0.619	0.588	5.00
		0.625	0.97
	0.0619	0.0600	3.07
		0.0600	3.07
5	0.619	0.613	0.97
		0.613	0.97
	0.0619	0.0600	3.07
		0.0625	0.97



electrode numbers 2,3,4 and 5 respectively. The straight line shown in Figure 14 is used as the calibration curve for the electrode number 1. Having found the amount of NH_4^+ produced, the concentration of urea is calculated from the stoichiometry of the reaction(8)and the results are summarized in Table 3. The relative error of the results obtained by this procedure is found to be less than 5%.

5-4. EFFECT OF SOAKING MEDIA ON THE RESPONSE CHARACTERISTICS.

In order to study the effect of soaking media on the response characteristics of the glass electrodes, the electrode numbers 2 to 5 were soaked in 0.1M NH_4Cl solution, pH 7.1 for a period of 24 - 82.5 hours, and then tested in the ammonium chloride solutions of concentrations 0.01 and 0.1M in that order. The results are summarized in Tables 4,5,6, and 7 respectively.

On comparing the results of Tables 4,5,6 and 7 with those of Figures 5,6,7 and 8 respectively, it is seen that the response of the electrodes is comparatively much faster and the magnitude of the e.m.f. drift is smaller, when they are preconditioned in 0.1M NH_4Cl , pH 7.1, rather than four times distilled water.

Although, the response is seen to be faster, preconditioning in NH_4Cl for more than 72 hours is found to result in large deviations of the order of 36.0-58.0 millivolts from the expected values of the steady-state e.m.f. and the response is observed to be non-Nernstian. However, from Table 7, it is noted that for the electrode soaked in 0.1M NH_4Cl , pH 7.1 for 24 hours only,

TABLE 4: TIME DEPENDENT RESPONSE OF THE ELECTRODE NO. 2,
SOAKED FOR 82.5 HOURS IN 0.1M NH₄Cl SOLUTION,
pH 7.1, TO NH₄⁺-IONS. TEMPERATURE = 28.0 ± 1.0°C
AND pH = 7.1 ± 0.1.

Time, minutes	e.m.f., millivolts	
	0.01 M NH ₄ ⁺	0.1 M NH ₄ ⁺
1.0	-41.0	-10.0
5.0	-42.0	- 3.0
10.0	-43.0	- 2.0
15.0	-44.0	- 1.0
20.0	-45.0	0.0
25.0	-46.0	0.0
30.0	-47.0	0.0
40.0	-48.0	+ 1.0
50.0	-48.0	+ 2.0
60.0	-48.0	+ 2.0
70.0	-	+ 2.0

TABLE 5: TIME DEPENDENT RESPONSE OF THE ELECTRODE NO. 3,
 SOAKED FOR 74.5 HOURS IN 0.1M NH₄Cl SOLUTION,
 pH 7.1, to NH₄⁺ IONS. TEMPERATURE = 28.0±1.0°C
 AND pH = 7.1 ± 0.1.

Time, minutes	e.m.f., millivolts	
	0.01M NH ₄ ⁺	0.1M NH ₄ ⁺
1.0	+109.0	+138.0
5.0	+104.0	+142.0
10.0	+102.0	+144.0
15.0	+102.0	+146.0
20.0	+102.0	+147.0
25.0	+102.0	+148.0
30.0	+102.0	+149.0
40.0	-	+150.0
50.0	-	+151.0
60.0	-	+152.0
70.0	-	+152.0

TABLE 6: TIME DEPENDENT RESPONSE OF THE ELECTRODE NO. 4,
SOAKED FOR 79 HOURS IN 0.1M NH₄Cl SOLUTION,
pH 7.1, to NH₄⁺ IONS. TEMPERATURE = 28.0 ± 1.0 °C
AND pH = 7.1 ± 0.1.

Time, inutes	e.m.f., millivolts	
	0.01 M NH ₄ ⁺	0.1M NH ₄ ⁺
1.0	-28.0	- 4.0
5.0	-33.0	0.0
10.0	-35.0	+ 2.0
15.0	-37.0	+ 3.0
20.0	-38.0	+ 4.0
25.0	-38.0	+ 5.0
30.0	-38.0	+ 6.0
40.0	-38.0	+ 8.0
50.0	-	+ 9.0
60.0	-	+ 9.0
70.0	-	+ 9.0

TABLE 7: TIME DEPENDENT RESPONSE OF THE ELECTRODE NO. 5,
SOAKED FOR 24 HOURS IN 0.1M NH₄Cl SOLUTION, pH
7.1, to NH₄⁺-IONS. TEMPERATURE = 28.0 ± 1.0°C
AND pH = 7.1 ± 0.1.

Time, Minutes	e.m.f., millivolts	
	0.01 M NH ₄ ⁺	0.1 M NH ₄ ⁺
1.0	+66.0	+126.0
5.0	+54.0	+122.0
10.0	+51.0	+120.0
15.0	+49.0	+120.0
20.0	+48.0	+119.0
25.0	+48.0	+119.0
30.0	+52.0	+119.0
40.0	+60.0	-
50.0	+61.0	-
60.0	+62.0	-
70.0	+62.0	-

the deviations from the expected steady-state e.m.f. values are of the order of 7.0 - 10.0 millivolts which are comparatively much smaller than the electrodes soaked for more than 72 hours and the response is observed to be nearly Nernstian in this case. These results suggest that there is a pronounced effect of soaking media and period of soaking on the response time. This interpretation is supported by the observations of Beck et.al.(68) who report that the pretreatment of the glass electrode in a medium similar to that it is to be used in, reduces time variations, and may alter the characteristic pattern of the time variation of potential, making precise measurements possible.

On the basis of these results, it is considered that there seems to be an optimum soaking time in 0.1M NH_4Cl , pH 7.1 that will lead to fastest response. It is felt that either by lowering the soaking time in 0.1M NH_4Cl , pH 7.1, or by storing the electrodes in a very dilute solution of ammonium chloride (probably 0.001M), it may be possible to reduce the response time considerably and get the Nernstian response.

All the electrodes were found to return to their original response when they were restored to four times distilled water and tested after 96 hours.

5-5 STABILITY OF THE ELECTRODES.

In order to study the stability of the electrodes, they were tested for their steady-state response in ammonium

TABLE 8: RESULTS OF STABILITY-STUDIES FOR VARIOUS
ELECTRODES IN NH₄Cl. TEMPERATURE=28.0 ± 1.0°C,
pH = 7.1 ± 0.1.

Electrode	Concentration of NH ₄ Cl, M	Steady-State e.m.f., millivolts		
		0 Day	6 Days	14 Days
1	1.0000	+174.0	+174.0	+173.0
	0.1000	+113.0	+113.0	+111.0
	0.0100	+ 54.0	+ 52.0	+ 51.0
	0.0010	- 13.0	- 10.0	- 12.0
	0.0001	- 72.0	- 70.0	- 73.0
2	1.0000	+ 24.0	+ 24.0	+ 23.0
	0.1000	- 34.0	- 34.0	- 35.0
	0.0100	- 92.0	- 93.0	- 93.0
	0.0010	-149.0	-150.0	-149.0
	0.0001	-209.0	-210.0	-210.0
3	1.0000	+174.0	+174.0	+174.0
	0.1000	+114.0	+114.0	+113.0
	0.0100	+ 53.0	+ 53.0	+ 54.0
	0.0010	- 9.0	- 9.0	- 9.0
	0.0001	- 69.0	- 69.0	- 69.0
4	1.0000	± 20.0	+19.0	± 20.0
	0.1000	- 38.0	-38.0	- 38.0
	0.0100	- 96.0	-97.0	- 96.0
	0.0010	-152.0	-152.0	-152.0
	0.0001	-212.0	-212.0	-212.0
5	1.0000	+189.0	+190.0	+189.0
	0.1000	+129.0	+129.0	+129.0
	0.0100	+ 69.0	+ 69.0	+ 70.0
	0.0010	+ 9.0	+ 9.0	+ 9.0
	0.0001	- 51.0	- 51.0	- 51.0

chloride solutions of various concentrations at different times and the results are shown in Table 8.

All the electrodes are found to be stable and producing excellent reproducible results.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6-1. CONCLUSIONS.

Based on the results of the present study, the following conclusions can be made:

1. The electrodes with sensing tips comprised of specially formulated glasses having compositions NAS27-3+ 3-mol% ZnO and NAS27-4 are sensitive to ammonium ions and give a Nernstian response to NH_4^+ in the concentration range 1.0 to 0.0001M. The experimental Nernst slopes agree within 5.19% of the theoretical value at 28°C.
2. Response time of the electrode depends on the direction of the concentration jump and the concentration of ammonium ions in the test solution. The response time decreases with decrease in ammonium ion concentration when the e.m.f. measurements are made in the sequence of more to less concentrated solutions, while on passing from less to more concentrated solutions, the response time decreases with increase in ammonium ion concentration.
3. The ammonium ion-sensitive glass electrodes can be used for the determination of urea via urease-catalyzed hydrolysis of urea to NH_4^+ with an accuracy of 5.00%.
4. The response rates of the glass electrodes are highly dependent on the type of soaking media in which the

electrodes are preconditioned. Pretreatment of the glass electrode in a medium similar to one it is to be used in, gives a faster response compared to preconditioning in four times distilled water.

6-2. RECOMMENDATIONS.

The following are some of the suggestions for further work.

1. On the basis of the present experimental results, an attempt should be made to derive a mathematical equation that could describe the e.m.f. - time behavior of these electrodes. Once such an equation is available, one can estimate the steady-state response from the initial response without waiting for the c.m.f. to come to its steady value, thus lowering the time of batch analysis considerably with the electrodes having high response times.

2. It is suggested that glass electrodes with bulbs having different wall thicknesses in the range 0.1 - 1.0 mm should be made and studied for the effect of membrane thickness on the response time.

3. In order to cut short the response time of the present electrodes, one should try to study the response times of these electrodes after pre-conditioning them either in ammonium chloride or ammonium hydroxide solution of various concentrations for different length of times. The results of such experiments can be used in framing the guidelines which will help in selecting

the proper soaking media and deciding the soaking time that would lead to fastest response.

4. The selectivity of the present glass electrodes to various monovalent cations, namely H^+ , Na^+ , and K^+ should be found by following the method of Esenman(28).

5. Using the procedure of Montalvo and Guilbault(41), it is suggested that urease electrodes should be prepared by placing a thin film of urease enzyme immobilized in acrylamide gel over the outer surfaces of the bulbs of the present glass electrodes and tested for their ready application to the determination of urea in blood and urine.

6. Finally, it is recommended that the output signal of the pH meter should be displayed on a potentiometric recorder in order to achieve high accuracy of the results.

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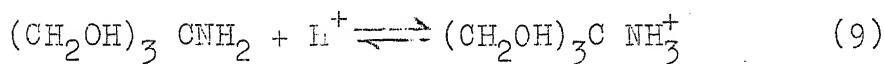
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APPENDIX

The primary amine tri-(hydroxymethyl)- aminomethane, or "Tris" has found extensive use as a buffer in biochemistry (69). This amine reacts with acid to form the corresponding salt of the amine:



The pK_a for the dissociation of the acid formed is 8.0.

Procedure for the Preparation of 500 ml of 0.1M Tris Buffer, pH 7.2:

In order to prepare the buffer solution, the ratio of the conjugate base to the acid in the buffer solution is determined first. This can be calculated by means of the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[\text{Base}]}{[\text{Acid}]} \quad (10)$$

By substituting the values of pH and pK_a into equation (10), one gets

$$7.2 = 8.0 + \log \frac{[(CH_2OH)_3\text{C NH}_2]}{[(CH_2OH)_3\text{CNH}_3^+]}$$

$$-0.8 = \log \frac{[\text{Free Amino}]}{[\text{Acid Salt}]}$$

$$0.8 = \log \frac{[\text{Acid Salt}]}{[\text{Free Amino}]}$$

$$6.31 = \frac{[\text{Acid Salt}]}{[\text{Free Amino}]}$$

This means that for every mole of free amine, there are going to be 6.31 moles of acid salt in the buffer solution.

Hence Tris buffer with this desired composition will have 86.32 mole percent ($\frac{6.31}{7.31} \times 100 = 86.32$) of the total buffer as the acid salt and 13.68 mole percent ($\frac{1}{7.31} \times 100 = 13.68$) as free amine. Since 500 ml of 0.1M buffer will contain 0.05 mole ($\frac{0.1}{1000} \times 500 = 0.05$) of Tris (salt and free amine), the buffer will contain 0.8632×0.05 or 0.043160 mole of acid salt and 0.1368×0.05 or 0.006840 mole of free amine.

Therefore, to prepare the buffer, 0.05 mole or 6.05 grams ($0.05 \times 121 = 6.05$) of solid amine (molecular weight = 121) were taken in a 500-cc volumetric flask, 0.043160 mole of HCl or 3.80 ml of 11.35 M HCl ($\frac{1000}{11.35} \times 0.043160 = 3.80$) added to it, and dissolved in four times distilled water. The contents were then diluted to the mark with four times distilled water.

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